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ASSESSMENT OF PAIN BY COMPARISON OF NALBUPHINE AND TRAMADOL WITH COMBINATION OF KETAMINE FOR OVARIOHYSTERECTOMY IN DOGS

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ABSTRACT

This study was conducted on eight (08) dogs weighing 9-22 kg, aged 2-3 years to evaluate the pain and efficacy of Nalbuphine and Tramadol with combination of Ketamine for ovariohysterectomy. It was hypothesized that Nalbuphine with combination of Ketamine would produce good analgesic effect as compared to Tramadol in dog model after ovariohysterectomy. Two groups of dogs were randomly selected. In group A, Nalbuphine 4 mg/kg bwt+Ketamine 10 mg/kg bwt were used and in group B, Tramadol 4 mg/kg bwt) + Ketamine 10 mg/kg bwt was given preoperatively. Ovariohysterectomy was performed as per standard technique through ventral midline approach by using all the aseptic precautions. Moreover, the numerical rating scale (NRS) was used for studying behavioural parameters for pain assessment. Results revealed in all two groups. The onset of anesthesia was faster in group B than in group A. There was no significant difference in the duration of anesthesia, rectal temperature, heart rate and respiratory rate between the two groups. However, the dogs in group B had significantly higher posture scores at 72 hours after surgery than the dogs in group A. The dogs in group B also had significantly higher appetite and thrust, personality and facial expression scores, and mental status scores at 24 and 72 hours after surgery, respectively. It is concluded that Nalbuphine with combination of Ketamine HCL showed better analgesic for ovariohystrectomy in dog. Further it is suggested that this experiment may be tested in cats.

INTRODUCTION.

An internal or external noxious stimulus is typically the cause of pain which is unconformable and emotional occurrence. It is an individual, subjective experience involving behavioral, emotional, and sensory elements related to existing or prospective tissue damage (Rawal, 2009). Animals perceive pain as a multifaceted physiological, sensory, and affective event, much like people do, despite their inability to articulate the emotional aspect that is particular to each individual animal. Pain can be triggered by a variety of methods because the pain pathway is pliable and changes and is modulated along its course rather than traveling straight from stimulation point to the perception site. Nevertheless, no single medication or method may adequately treat every type of pain. This makes the doctor aware that managing pain requires a multimodal, or "balanced," analgesic strategy. (Zaki, 2013).

Although exact understanding and identification of the assessing of pain is existence agonized (Sharkey, 2013). Meanwhile, the influence of post-surgical pain assess is difficult in dogs in the absence of vocal communication. As a result, the assessment of pain in veterinary medicine is affected by various factors such as, nature, vocalization, position, movement level, motion, response to palpation and other behavioural variations. It should be noted that pain thresholds and responses vary by species, breed, health and age (McMillan, 2016).

Opoids are morphine-like substance which bind with opoid receptors and start the pain by acting on receptors in the dorsal horn of spinal cord and mesolimbic system (Benson, 2002). There are several different receptors like kappa, mu and delta (Heavner and Cooper, 2008).

Nalbuphine is agonist-antagonist opoid that is competitive u-receptor antagonists but maintain their analgesic effects by acting on k receptors, Moreover, Nalbuphine induces mild analgesia with sedation and miosis (Benson, 2002).

Whereas, tramadol hydrochloride is a synthetic analgesic medicine that acts on the central nervous system. It is an opioid agonist that was permitted by the Food and Drug Administration (FDA) in 1995 for the management of pain (Casella et al., 2013). It is a safe medicine that utilized as a centrally acting pain-relieving. It did not show any of the side effects associated with typical opioids, however, reported tramadol toxicity and misuse is prospective (Tjäderborn et al., 2007).

While, ketamine is a dissociative anesthetic drug mostly used in the veterinary anesthesia. It provides extreme analgesia (Rogers, 2020). It inhibits the N-methyl-D-aspartate (NMDA) receptor has currently produced excessive excitement in the way of depression as a fast-acting antidepressant. One dose of ketamine provides an antidepressant effects within

hours of administer that continues for about one week (Berman et al., 2000). Ovariohysterectomy (OHE) of dogs and cats is one of the most frequently performed procedures in veterinary practice due to its possible advantages, for example control of population, avoid of reproductive tract diseases, reduction of unwanted activities related with hormonal activity and decrease stray and incontrollable populations of dogs and cats (DeTora & McCarthy, 2011). According to Robertson (2003), the N.R.S. (Numerical Rating Scale) worked best when all evaluations were completed by a single, qualified observer. It is acknowledged that preventing and treating pain are crucial components of comprehensive patient care.

Limited comparative research has been conducted on Nalbuphine and Tramadol to reduce pain strategy after ovariohysterectomy in dogs. Therefore, this study is designed to compare the analgesic effects of Nalbuphine and Tramadol in dogs after ovariohysterectomy. It is hypothesized that Nalbuphine would produce good analgesic effect than tramadol in dog model after ovariohysterectomy.

MATERIAL AND METHODS

This study was conducted on eight apparently healthy adult female dogs. They were trapped from Tandojam and brought at the indoor patient ward of Department of Surgery and Obstetrics, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. These dogs were fed and handled individually for a period of 14 days before the start of the experiment to adapt to the environment. During this period all dogs were subjected to clinical examination to rule out the pregnancy diagnosis and other disease conditions. Only healthy and non-pregnant dogs were selected for this study. The study was approved by Ethical Care Committees.

All the selected dogs were assigned into two groups as group A (Nalbuphine+ Ketamine group) and group B (Tramadol+ Ketamine group) using a complete randomized design comprising of four dogs each (Table-1).

Table 1 Experimental Design

Groups	**No. of Animals**	**Treatment**	**Dose (mg/kg)**	**Procedure**
A	04	Inj. Nalbuphine Inj. Ketamine	4 mg/kg bwt 10 mg/kg bwt	OHE
B	04	Inj.Tramadol Inj. Ketamine	4 mg/kg bwt 10 mg/kg bwt	OHE
Total	**08**			

Pre-operative preparation

The food was withheld 12 hours before to administration of anesthesia to avoid any complications. The surgical area for laparotomy was prepared using hair clipper machine with blade No: 40 (Oster, USA). The debris was removed by applying normal saline and finally antiseptic solution was applied. The incision site was aseptically prepared by application of hydrogen peroxide and Tincture Iodine.

Anesthesia and Analgesia

All the animals were given pre-emptive analgesia with Nalnuphine and Tramadol in respective groups. 15 minutes later atropine sulphate administered 0.04 mg/kg IM as premedication in dogs. The induction was achieved 15 minutes after atropine sulphate by xylazine-ketamine combination @ 2mg/kg and 10mg/kg respectively; half dose by IM and rest half by IV route. In group A dogs received Nalbuphine @ 4 mg /kg IM 15 minutes before the atropine sulphate. Similarly dogs of group B, Tramadol was given @ 4 mg/kg IM 15 minutes prior to atropine sulphate.

Surgical procedure for Ovariohysterectomy

The anesthetized dogs were placed on dorsal recombency on V-tray and then the surgical site was scrubbed with povidone and the operation site was covered with laparotomy sheet. Identified the umbilicus and ventral midline incision was given just caudal to the umbilicus. Incision was created 6 to 8 cm long through skin and subcutaneous into the abdominal cavity. Then laparotomy incision extends the linea alba (ventral rectus sheath) and tented it outer and stab incision was made into the abdominal cavity. There surgical incision was extended to the linea alba incision caudal and cranial to the stab incision with mayo scissors. Afterward incision was elevated the left abdominal wall by grasping the external rectus sheath with thumb forceps. Then ovariectomy hook was slide against the abdominal wall, 2 to 3 cm caudal to the kidney. Then turn hook turn medially to ensure the uterine horn and gently elevate it from abdomen. Suspensory ligament was break near the kidney to allow exteriorization of the ovary. Rochester-carmalt forcep was applied for ligation of blood and placed a figure-eight ligature proximal to the ovarian pedicle clamps with vicryl (2-0) and transected the ovary. Place the ligation suture circumferential near cervix. Then placed forceps cranial to the ligatures and transected the uterine body (Plate-3.6). Abdominal wall was closed by three layer peritoneum muscles and subcutaneous tissue with vicryl (2-0) by continuous suture technique and skin was closed with silk (2-0) by simple interrupted suture technique.

Post-operative care:

The laparotomy wound was cleaned with hydrogen peroxide to remove blood spots. Povidone iodine and CTC spray were then applied to the wound for disinfection. To prevent secondary

infection, injections of amoxicillin were administered on every 24 hours for a total of 5 days. Analgesics (Tramadol and Nalbuphine) were provided for pain management. A soft diet was offered to the dogs until complete wound healing.

Parameters

The following parameters were recorded during the whole experiment.

Effect of Anesthesia (minutes)

During the whole of the experiment, the dogs were closely monitored after every five minutes for recording the various parameters. i.e., the onset of anesthesia, duration of anesthesia

Other observation

Other parameters that were studied such as 1. Salivation, 2. Jaw tune, 3. Protrusion of tongue, 4. Urination, 5. All parameters were recorded after every 5 minutes interval of during anesthesia.

1. Physiological parameters

Rectal temperature, pulse rate (beat per minute), respiratory rate (breath per minute) were recorded after complete recovery, 24 hrs, 48 hrs and 72 hrs postoperatively.

2. Behavioral parameter

The responses of behavioral were monitored after administration of analgesic drugs follow by after complete recovery, 24 hrs, 48 hrs and 72 hrs respectively. The assessment of pain was performed by using multifactorial rating scale (NRS). The pain assessment was made by using a multi factorial numerical rating scale (NRS). There are seven behaviors used in this scale such as, posture behavior, vocalization, appetite and thrust, response to palpation, facial expression and mental status. All these parameters except appetite and thrust were graded by multifactorial score from 0-3 (table 2).

Table 2 Numerical Rating Scale

Behavioral parameters	Scale	Observations
Posture	**0**	Sitting or standing head up
	1	Lateral recumbency
	2	Restlessness
	3	Tucked up appearance
Vocalization	**0**	No vocalization
	1	Vocalization when forced to move
	2	Vocalization when touched
	2	Intermittent
	3	Continues

Appetite & Thrust	**0**	Taking food and water
	1	Taking liquid only
	2	Not taking any thing
Personality/Attitude	**0**	At rest
	1	Quiet and docile animal may become aggressive
	2	Licking/biting/scratching the painful area
	3	Self-mutilation
Response To Palpation	**0**	No change
	1	Guards/reacts when touched
	2	React before touched
	3	Severe response
Facial Expression	**0**	Active
	1	Dull eyes
	2	Staring in space
	2	Appears sleepy
	3	Photophobic appearance
Mental Status	**0**	Submissive
	1	Overfriendly
	2	Wary
	3	Aggressive

Biochemical and Hematological parameters

Haematological parameters were estimated in approximately 0.5ml of blood in EDTA vial using auto analyzer machine (Haematology Cell Counter MS4, France).

Total leukocyte (TLC), Hemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC). Blood biochemical examination were performed by standard procedure using commercially available kits (spectrophotometer analyzer using biosystem BTS-350 kits) Glucose was estimated in plasma collected in 3.8% sodium fluoride solution.

Following parameters were estimated in the serum samples.

Glucose, Creatinine, Blood urea nitrogen (BUN), Albumin, Cortisol and C-reactive proteins (CRP).

Statistical analysis

The recorded data was statistically analyzed used to detect the effect of factor (group) in study parameters. Data was collected on proforma presented in mean and standard error. Analysis of variance was performed.

RESULTS

Anesthesia

The mean ± S.E values for onset of anesthesia were 1.75±0.25 and 1.5±0.28 minutes in both group A and B individually observed in all operated dogs during ovariohysterectomy procedure. After administration of anesthesia the onset of anesthesia was rapid in (group B) as compare to (group A). While, the mean ± S.E values for duration of anesthesia a took slightly more duration of anesthesia (53.50±2.21) minutes as compared to the group B (50.75±0.47) minutes (Table 3).

Table 3 Onset of anesthesia (minutes) in the both group of dogs.

Parameter	Group A	Group B
Onset of Anesthesia (minutes)	1.75±0.25[a]	1.5±0.28[a]
Duration of anesthesia (minutes)	53.50±2.21[a]	50.75±0.47[a]

Means with different superscripts vary significantly (p<0.05) between groups.

Other Observation

Other parameters and reflexes were observed during the whole study e.g, salivation, urination anal relaxation, analgesia, corneal reflexes, pedal reflexes, jaw tone reflexes and tongue pinch reflex. In this study Salivation was occurred in 2 of 4 Dogs in (group A) and 3 of 4 Dogs in (group B) while urination was present in 2 of 4 Dogs in group B (Table 4).

Table 4 Summary of reflexes of the both group A and B in Dogs

Reflexes	Treatment	
	Group A	Group B
Rexumbency	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

Physiological Parameters

The mean values of rectal temperature score were found lower in dogs under group A at after complete recovery of ovariohysterectomy. Moreover, the mean values of score were observed non-significantly variation at various time interval in the dogs of both groups and score are fluctuated in between (70.83±0.31 to 72.83±0.60) (Table 5). Although, the mean values of respiratory rate score were fluctuated within normal physiological limits in all the groups of animals during the present study.

Table 5. Mean values of Physiological parameters under Nalbuphine and Tramadol group of dogs.

Parameters	**Groups**	**After complete recovery**	**24hrs**	**48hrs**	**72hrs**
Rectal temperature	**Group A**	97.83±1.48^{a}	102.38±0.24^{a}	102.30±0.12^{a}	102.38±0.24^{a}
	Group B	100.18±0.83^{a}	102.00±0.35^{a}	101.75±0.14^{a}	102.38±0.13^{a}
Heart Rate	**Group A**	72.33±0.21^{a}	72.32±0.21^{a}	70.83±0.31^{a}	72.83±0.60^{a}
	Group B	72.00±0.31^{a}	71.61±0.42^{a}	72.83±0.60^{a}	72.17±0.48^{a}
Respiratory rate	**Group A**	22.00±0.41^{a}	23.00±1.08^{a}	24.25±0.75^{a}	24.25±0.29^{a}
	Group B	21.00±0.58^{a}	24.75±1.11^{a}	25.00±0.00^{a}	25.00±0.50^{a}

Means with different superscripts vary significantly ($p<0.05$) between

Behavior pain assessment

The mean values of posture score were found significantly higher (<0.05) for the posture of dogs under group B after 72hrs of ovariohysterectomy compared to that of after 24hrs and after complete recovery. Moreover, the mean values of vocalization and response to palpation score were observed non-significantly variation at various time interval in the dogs of both groups and score are fluctuated in between (0.25±0.25 to 1.5±0.6) and (0.25±0.25 to 1.25±0.47). The mean values of appetite thrust, personality/attitude and facial expression score were found significantly higher (<0.05) for the of dogs under group B after 24hrs.

Additionally, the mean values of mental status score were found significantly higher (<0.05) of dogs under group B after 72hrs of ovariohysterectomy compared to that of after 24hrs and after complete recovery (Table 6).

Table 6 Mean values of Behavior under Nalbuphine and Tramadol group of dogs

Parameters	**Groups**	**After complete recovery**	**24hrs**	**48hrs**	**72hrs**
Posture	**Group A**	0.0 ± 00^{b}	0.25 ± 0.25^{ab}	1.5 ± 0.9^{ab}	1.00 ± 0.7^{ab}
	Group B	0.5 ± 0.29^{ab}	1.5 ± 0.29^{ab}	1.00 ± 0.7^{ab}	1.75 ± 0.47^{a}
Vocalization	**Group A**	0.25 ± 0.25^{a}	1.00 ± 0.4^{a}	1.5 ± 0.5^{a}	1.0 ± 0.57^{a}
	Group B	0.75 ± 0.25^{a}	1.00 ± 0.57^{a}	1.25 ± 0.4^{a}	1.5 ± 0.6^{a}
Appetite and thrust	**Group A**	0.5 ± 0.29^{ab}	0.5 ± 0.29^{ab}	0.25 ± 0.25^{ab}	0.00 ± 0.00^{b}
	Group B	1.00 ± 0.4^{b}	2.00 ± 0.4^{a}	0.5 ± 0.29^{ab}	0.25 ± 0.25^{ab}
personality/attitude	**Group A**	0.5 ± 0.29^{b}	1.25 ± 0.25^{ab}	0.75 ± 0.47^{ab}	0.5 ± 0.29^{b}
	Group B	0.75 ± 0.25^{ab}	1.75 ± 0.25^{a}	1.00 ± 0.7^{ab}	1.5 ± 0.29^{ab}
Response to palpation	**Group A**	0.5 ± 0.29^{a}	0.75 ± 0.25^{a}	0.25 ± 0.25^{a}	0.25 ± 0.25^{a}
	Group B	0.5 ± 0.29^{a}	1.25 ± 0.47^{a}	1.00 ± 0.7^{a}	1.00 ± 0.7^{a}
Facial expression	**Group A**	1.00 ± 0.4^{ab}	1.5 ± 0.29^{ab}	0.25 ± 0.25^{b}	0.5 ± 0.5^{ab}
	Group B	1.00 ± 0.00^{ab}	2.00 ± 0.00^{a}	1.00 ± 0.5^{ab}	1.00 ± 0.57^{ab}
Mental status	**Group A**	0.5 ± 0.29^{b}	1.00 ± 0.00^{ab}	1.00 ± 0.00^{ab}	1.00 ± 0.00^{ab}
	Group B	0.75 ± 0.25^{ab}	1.00 ± 0.4^{ab}	1.5 ± 0.5^{ab}	1.75 ± 0.47^{a}

Means with different superscripts vary significantly ($p<0.05$) between groups.

Legend: - Score: **Posture** 0-Sitting or standing head up 1- Lateral recumbency 2-Restlessness 3-Tucked up appearance **Vocalization** 0-No vocalization 1-Vocalization when forced to move 2-Vocalization when touched 2-Intermittent 3-Continues **Appetite and Thrust** 0-Taking food and water 1- Taking Liquid only 2-Not Taking anything. **Personality/attitude** 0- At rest 1- Quite and docile animal may become aggressive 2- Licking/biting/scratching the painful area 3- Self mutilation. **Response to palpation** 0- No change 1- Guards/reacts when touched 2- React before touched 3- Severe response. **Facial expression** 0- Active 1- Dull eyes

2- Staring in space 2- appears sleepy 3- Photophobic appearance **Mental status**. 0- Submissive 1- Overfriendly 2- Wary 3- Aggressive

Biochemical and Hematological parameters

The data illustrates changes in various health markers at different time intervals (24, 48, and 72 hours) following complete recovery for two groups, Group A and Group B. Group A experienced a consistent decrease in glucose levels over time, whereas Group B showed a similar trend but with higher values. Creatinine and BUN levels were consistently higher in Group B throughout the recovery period. Group A had higher albumin levels, while C-reactive protein (CRP) levels decreased in both groups. Cortisol levels fluctuated differently in both groups. Hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), and different types of white blood cells showed varying patterns. Notably, some parameters exhibited significant differences between the two groups at specific time points. In summary, the data indicates dynamic changes in health markers during the recovery period, with potential group-specific variations (Table 7).

Table 7 Biochemical and Hematological Parameters of Nalbuphine and Tramadol group of dogs

Parameters	**Groups**	**After complete recovery**	**24hrs**	**48hrs**	**72hrs**
Glucose (mg/dl)	**Group A**	83.20 ± 1.36^{b}	81.14 ± 1.36^{b}	72.12 ± 0.63^{ab}	62.10 ± 0.52^{a}
	Group B	102.40 ± 1.80^{b}	98.24 ± 2.08^{b}	95.36 ± 1.68^{b}	81.76 ± 1.62^{a}
Creatinine (mg/dl)	**Group A**	$1.31.14\pm0.02^{b}$	1.25 ± 0.03^{b}	1.23 ± 0.02^{b}	1.14 ± 0.01^{a}
	Group B	1.49 ± 0.06^{a}	1.48 ± 0.06^{a}	1.50 ± 0.04^{a}	1.42 ± 0.05^{a}
Bun (mg/dl)	**Group A**	13.24±0.48	13.23±0.46	13.08±0.45	12.42±0.24
	Group B	11.50±0.15	11.74±0.46	11.92±0.47	10.19±0.26
Albumin (g/dl)	**Group A**	2.13 ± 0.22^{a}	2.11 ± 0.16^{a}	2.17 ± 0.24^{a}	2.40 ± 0.36^{b}
	Group B	1.74 ± 0.15^{a}	1.64 ± 0.08^{a}	1.72 ± 0.08^{a}	1.61 ± 0.24^{a}
CRP (mg/dl)	**Group A**	2.14 ± 0.12^{b}	1.86 ± 0.04^{b}	1.82 ± 0.02^{b}	1.64 ± 1.13^{ab}
	Group B	1.51 ± 0.12^{b}	1.56 ± 0.04^{b}	1.66 ± 0.06^{ab}	1.71 ± 0.02^{b}
Cortisol (µg/dl)	**Group A**	6.01 ± 0.44^{b}	6.59 ± 0.44^{b}	5.54 ± 0.36^{a}	2.23 ± 0.52^{a}
	Group B	6.74 ± 0.18^{b}	6.98 ± 0.18^{b}	4.18 ± 0.23^{b}	3.23 ± 0.21^{a}
Hb (g/dl)	**Group A**	10.97±0.71	10.92±0.63	11.05±0.70	11.54±0.68
	Group B	12.44±0.93	12.52±0.87	12.65±0.85	12.80±0.88
PCV (%)	**Group A**	40.56±1.55	39.89±1.65	43.14±1.56	43.18±1.55
	Group B	36.32±1.59	36.23±1.46	38.14±1.49	38.06±1.78

TEC (million/ µl)	**Group A**	5.21±0.36	5.14±0.36	4.94±0.34	5.21±0.36
	Group B	5.87±0.36	5.86±0.32	5.98±0.40	6.05±0.24
TLC (no. / µl)	**Group A**	17682±1950	17771±1980	16281±1920	16660±1840
	Group B	18525±22332.98[a]	18625±1463.76[a]	25333.2±2416.16[b]	18366.7±2995.1[a]
Neutrophils (%)	**Group A**	74.40±0.91[b]	75.96±0.65[b]	64.20±0.71[a]	62.81±0.66[a]
	Group B	63.60±0.92[a]	64.00±0.36[a]	75.80±0.58[b]	77.80±0.73[b]
Monocytes (%)	**Group A**	1.00±0.63	1.40±0.81	1.60±0.68	1.20±0.46
	Group B	2.60±0.68[a]	2.60±0.67[a]	2.72±0.87[a]	2.21±0.66[a]
Eosinophil (%)	**Group A**	6.00±0.51	5.60±0.36	4.80±0.55	5.20±0.96
	Group B	0.80±0.48[a]	0.60±0.40[a]	0.40±0.40[a]	0.60±0.41[a]
Lymphocytes (%)	**Group A**	21.20±1.06	18.56±1.32	17.76±1.36	81.14±1.36
	Group B	25.42±1.06[a]	24.60±1.50[a]	23.60±1.10[a]	31.40±0.92[b]
Basophils (%)	**Group A**	0	0	0	0
	Group B	0	0	0	0

Means with different superscripts vary significantly ($p<0.05$) between groups

DISCUSSION AND CONCLUSION

In current study, mean values for onset of anesthesia were 1.75±0.25 and 1.5±0.28 minutes in A and B group respectively, observed in all operated dogs during OHE procedure. In group B the onset of anesthesia was rapid as compare to group A. However, related results were observed by Ajadi et al. (2009), they mentioned that Tramadol+Ketamine had provide rapid onset, smooth induction and safe recovery with less side effects and better respiratory status. Present study showed that the values for duration of anesthesia was monitored in all operated dogs during OHE procedure. It was obvious from the data, that the dogs in group A, took slightly more duration of anesthesia (53.50±2.21) minutes as compared to the group B (50.75±0.47) minutes. However, same related findings were also observed by Abouelfethouh et al, (2022). They found that Nalbuphine+Ketamine combination produced acceptable and better quality induction and duration of anesthesia.

In ongoing study, the score of rectal temperature (0F) was decreased in animals under group A, and values were fluctuated within (97.83±1.48 to 102.38±0.13) after complete recovery of OHE as compared to that of 24hrs 48hrs and 72hrs. However, similar relationship were observed by Buhari et al, (2012) and they found that the factor monitored and it showed intensity of painful state no any noticeable physiological effect in animals.

Mean values of heart rate (beat per minute) were observed non-significantly variation at different time interval in the dogs and mean values were changed between (70.83±0.31 to 72.83±0.60) from after complete recovery, 24hrs, 48hrs and 72hrs. While, similar result determined by Derossi et al, (2015) and buhari et al. (2012). They found that heart rate and pressure of blood were maintained within renascence limit.

The present study shows that the mean values of respiratory rate (breath per minute) score were fluctuated within normal physiological limits in all the groups of animals during the present study. However, Related findings obtained by Derossi et al. (2015) and Buhari et al. (2012). They observed nalbuphine and tramadol produced limited side effects on respiratory rate.

Results of current study showed that total mean values of posture score was found higher in dogs under group B and the values varied between (0.0±00 to 1.75±0.47) after 72hrs of OHE compared to that of subsequently 24hrs and after complete recovery. Our finding are in agreement with Alonso et al. (2005) and Gupta et al. (2009), they concluded that less pain score variation like standing head down and lateral recombency detected more than 24 hrs post surgical period of time only variation in posture such as appearance of tucked up is not clear evidence in any dogs.

Whereas, vocalization mean score was monitored non significantly fluctuation at various time period in dogs of both group and mean values were moves between (0.25±0.25 to 1.5±0.6) from after complete recovery, 24hrs and 72hrs. However, in vocalization parameter when touch to operative site was observed in both groups. Meanwhile, vocalization is about rarely useful sign of pain in animals Watson et al. (1996). Even so, this may be also indication of anxiety, anesthesia evoked fear Conzemius et al. (1997). Therefore, these sign is intensive and non-particular sign of pain (fazili, 2005 & Metha, 2006).

Furthermore, mean score of thrust and appetite appetite and thrust score were found significantly higher in dogs under group B and the score values turned between (0.5±0.29 to 2.00±0.4) after 24hrs of OHE compared to that of after 48 and 72hrs. While, these observations were supported by similar results of Orr et al. (2005), starvation condition were normal within one or two days postoperatively. However, Kohn et al. (2013) reported that the appetite and thrust, considered with stool production and urine did not effected. However, prolonged stress suppress the intake of feed, this parameter are according to poor signs of pain Hellyer, (1999).

Whereas, in present study the mean values of attitude score were found significantly higher in dogs under group B and the mean values multifaceted between (0.5 ±0.29 to 1.75±0.25) after 24hrs of OHE compared to that of after 48 and 72hrs. It might be because of

post-surgical pain and its degree level is higher in post-surgical time. Observation were similar to Gupta et al. (2009). Moreover, in result of current study the mean score of response to palpation score were observed non-significantly variation at different interval of time in the both group of dogs, the score were turned between (0.25±0.25 to 1.25±0.47) from after complete recovery, 24hrs and 72hrs. These observations were supported by similar results of Fazili et al. (2008) & Watson et al. (1996).

Meanwhile, the current study the mean values of facial expression score were originated significantly higher in dogs under group B and the score values fluctuated between (1.00±0.4 to 2.00±0.00) after 24hrs of OHE compared to that of after 48 and 72hrs. The both groups were calm and submissive, some were stressed in facial expression, some dogs of group A and B appears sleepy, dull eyes and staring in space. Moreover, similar finding reported by Robertson, (2003) determined dilated pupils, dull eyes, and sleepy appearance to be indicator of pain. In addition, in dogs expression of face could be helpful for to indicate the pain Roberstson, (2002). The score of mental status were found significantly higher in dogs under group B and the score values moved between (0.5±0.29 to 1.75±0.47) after 72hrs of OHE compared to that of after 24hrs and after complete recovery. Similar results were observed by (Fazili et al. 2008 and Gupta et al. 2009). Meanwhile, some of few behaviours were examined in this study,the higher mean score were found in facial expression, response to palpation, appetite and thurst and vocalization emotions in dogs of both groups during postoperative periods respectively.

Furthermore, reaction to touch seemed expected individual one of the good sign of pain. These recommendation were similarly the findings of Deneuche, (2004) & Fazili, (2005) they further claimed that mental state,touch response and response to pressure are excellent indicator of pain.

Moreover, Significant variations in glucose levels were observed in both groups, with a noteworthy decrease in Group A at 72 hours compared to the baseline. Conversely, Group B displayed a more fluctuating pattern. These findings may suggest differential effects on glucose homeostasis, warranting further investigation into the mechanisms underlying these variations. Amal et al (2016) agree with findings. These findings suggest that tramadol use may have an impact on glucose homeostasis in dogs, and monitoring of glycemic and lactate levels is important in dogs treated with opioids.

Creatinine levels exhibited a consistent decreasing trend in Group A, indicating a potential positive influence on renal function. In contrast, Group B displayed marginal changes, suggesting a different response to the treatment regimen. BUN levels remained

relatively stable in both groups, indicating a limited impact on renal nitrogen excretion. . Similar findings were demonstrated by Costa et al. (2013). The serum urea levels were markedly elevated following Tramadol HCl administration but renal dysfunction was not expected because serum creatinine concentrations showed no significant changes. This was further supported by the results of creatinine clearance which showed no significant alterations after Tramadol HCl administration. Possible clarification and explanation for the improve in serum urea levels is the significant upswing in arterial blood pressure with a consequence decrease in renal blood flow, glomerular filtration rate and tubular excretion of urea. On contrary, Mcmillan et al. (2008) recorded no significant changes in blood biochemical parameters in dogs of the veins injected with Tramadol HCl at doses of 1, 2, and 4 mg/kg.

Albumin levels, shown significant vacillation in both groups. Group A exhibited an increase, while Group B displayed a decline over time. C-reactive protein (CRP) levels, indicative of inflammation, established a gradual decrease in both groups. These findings underscore the complex interplay between treatment interventions and inflammatory. Both nalbuphine and tramadol showed significant fluctuations in albumin levels in the studies. In a study by Sathyan et al., (2022) the side effects of nalbuphine and tramadol were compared in patients undergoing surgeries.

Cortisol levels, reflecting the endocrine response to stress, showed notable variations between the groups. Group A displayed a gradual decline, potentially indicating a reduced stress response, while Group B exhibited a more erratic pattern. Further exploration into the specific mechanisms influencing cortisol levels is warranted. These findings suggest that it can only be assumed that stress exists since cortisol is a very unspecific hormone and could be altered due to stress related to pain, but also due to fear, cold or even the anesthesia itself. Tissue injury leads to the activation of nociceptive and inflammatory responses that are often associated with pain and hyperalgesia and behavioral changes (Henson, 2021).

Hemoglobin (Hb) levels and hematocrit (PCV) remained relatively stable in both groups, suggesting minimal impact on red blood cell indices. Total leukocyte count (TLC) in Group B demonstrated a significant increase at 48 and 72 hours, indicating a potential immune response to the treatment regimen. These findings similar with (Rashmi, Salhotra et al., 2023). He reported that Nalbuphine HCl had no significant effects on RBCs, PCV, and Hb values. Differential effects on immune cell populations were observed, particularly in neutrophil and lymphocyte percentages. Group A showed a decrease in neutrophils and an increase in lymphocytes, while Group B exhibited the opposite trend. These variations may suggest distinct immunomodulatory effects of the treatments. Similar findings observed by Sayed

(2018). It is concluded that Nalbuphine produced longer duration analgesia as compared to tramadol and nalbuphine found to be better and more effective for ovariohystrectomy in post-operative pain management when compared to tramadol because, nalbuphine group of dogs had less surgical stress.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

This section should be filled with the initials of the authors specifying their contribution to the final form of the manuscript. If there is more than one author that participated in the sections metioned below, separate their initials by comma(s) (,).

Concept – AM, KAS.; Design – AM; Supervision – TAN, KAS, MBB; Resources - MA; Materials – AN; Data Collection and/or Processing – SLA; Analysis and/or Interpretation – AM; Literature Search – AM; Writing Manuscript – AM; Critical Review – LL.

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