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Comparative studies on haemato-biochemical changes following pre-emptive analgesia with tramadol, pentazocine lactate and meloxicam in pain management of canine ovariohysterectomy

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ABSTRACT

The present study was conducted to evaluate the effects of tramadol, pentazocine lactate and meloxicam as pre-emptive analgesics in dogs premedicated with glycopyrrolate, inducted with propofol and maintained with propofol continuous rate infusion (CRI) for certain haematological and biochemical parameters. The animals were randomly divided into three equal groups,viz. Group-T, Group-P and Group-M comprising six animals in each group and all the animals were premedicated with glycopyrrolate. After 10 min of pre-anaesthetic administration, pre-emptive analgesia was given. Blood was collected from cephalic or saphenous vein at intervals 0 (baseline) min before premedication, thereafter at 10 min, 30 min, 1 h, 2 h and 3 h after pre-emptive analgesic administration and haemato-biochemical parameters were recorded. Hb, PCV and TEC were significantly decreased at 30 min and 1 h interval in all the three groups. TLC and glucose concentration were significantly higher in group-M as compared to group-T and group-P at different time intervals. GGT level increased significantly at 30 min in all the three groups. CRP concentration was significantly higher in group-M as compared to group-T. Total protein was significantly decreased at 1 h interval in group-T and group-P, but in group-M such finding was noticed at 2 h interval. Cortisol was significantly lower in group-T in entire study period. The alterations in physiological and haematological parameters caused by tramadol, pentazocine lactate and meloxicam were found to be minimal and within the physiological limits. Tramadol produced less significant rise in CRP and cortisol concentrations which indicated better pain management. Based on the findings of the present study, it is concluded that tramadol is more effective as compared to pentazocine lactate and meloxicam in the management of post-operative pain due to canine ovariohysterectomy.

Keywords: Continuous rate infusion (CRI), Glycopyrrolate, Meloxicam, Pre-emptive analgesics, Pentazocine lactate, Propofol, Tramadol

All the surgical procedures, including ovariohysterectomy causes pain. Elective ovariohysterectomy is one of the most common surgical procedures carried out in general veterinary clinical practice of companion animals such as dogs and cats (Fox *et al*. 2000). The centre for Veterinary Medicine of the United Sates Food and Drug Administration (USFDA) consider ovariohysterectomy to cause moderate pain making it suitable for clinical studies of analgesia (Connolly 2002). Pain noticed in the post-operative period is classified as acute (Ready 1993) and is characterized by stressful emotional and sensory experiences. Pain management in animals depends upon recognition and assessment of pain, which can be notoriously difficult because of the complexity of pain physiology and an inability to verbally describe symptoms of pain. Therefore, initiation of analgesic therapy must depend upon behavioural observations, which is essential in pain assessment. Pre-emptive analgesia, the concept of which originated during the time of growing appreciation of dynamic characteristics of pain pathway, is the administration of effective analgesia prior to the surgical trauma (Kotur 2006). Tramadol is a centrally acting synthetic 4-phenyl-piperidine analogue of codeine (Lewis and Han 1996). Since endogenous norepinephrine and serotonin are involved in central pain modulation, these properties may thus enhance the analgesic effects of tramadol produced by its opioid binding activity. Pentazocine is a narcotic analgesic with mixed agonist and antagonist activity (Ram *et al.* 2016). Pentazocine has been used in the management of mild to moderate post-surgical pain. Meloxicam is a potent analgesic in abdominal surgical procedures of dogs and cats (Mathews *et al*. 2001, Slingsby and Waterman-Pearson 2000). Propofol can be used for both induction and maintenance of anaesthesia

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and because of its depressant effect on CNS, rapid action, excellent hypnosis, good muscle relaxation, non-cumulative effect and rapid complete recovery, it is most suitable for total intravenous anaesthesia (TIVA) (Adetunji *et al.* 2002). This study was undertaken to compare the efficacy of pre-emptive analgesia with Tramadol, Pentazocine lactate and Meloxicam in bitches undergoing ovariohysterectomy based on haemato-biochemical parameters.

MATERIALS AND METHODS

Experimental animals and preparation: The study was conducted in 18 clinical cases of dogs brought for ovariohysterectomy aged 1-4 years having body weight ranged from 9-15 kg. All animals were fasted overnight with food and water with-held for 12 and 6 h, respectively prior to the anaesthetic trial. Animals were examined for all the vital physiological parameters before any medication on the day of surgery.

Experimental design: The animals were randomly divided into three equal groups, viz. Group-T, Group-P and Group-M comprising six animals in each group and all the animals were premedicated with glycopyrrolate @0.01 mg/kg body weight, IM. After 10 min of pre-anaesthetic administration, pre-emptive analgesia was given (Tramadol @2 mg/kg, body weight, IV in Group-T, Pentazocine lactate @2 mg/kg, body weight, IV in Group-P and Meloxicam @0.2 mg/kg, body weight, IV in Group-M). After 10 min of pre-emptive analgesic administration, induction was achieved with propofol @5 mg/kg body weight, IV and maintained with the help of propofol @25 mg/kg/hr IV by CRI method up to 1 h.

Haematological parameters: The blood samples were collected from cephalic or saphenous vein in sterile vials containing dipotassium ethylene diamine tetra acetic acid (K2-EDTA) prior to pre-medication at 0 min (base line), thereafter 10 min, 30 min, 1 h, 2 h and 3 h after analgesic administration. Different haematological parameters such as haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count (granulocytes, lymphocytes and monocytes) were measured using automated haematology cell counter (MS4-e France).

Biochemical parameters: The blood samples were collected from cephalic or saphenous vein in sterile vials containing clot-activator vials prior to pre-medication at 0 min (base line), thereafter 10 min, 30 min, 1 h, 2 h and 3 h after analgesic administration. The blood was allowed to clot and the serum was separated by centrifugation at 4000 rpm for 10 min, and isolated serum samples were immediately used for the analysis of different parameters such as serum glucose using commercially available glucose estimation kit in Evolution 201 UV-Visible spectrophotometer by glucose oxidase-peroxidase (GOD-POD) method, gamma-glutamyl transferase (GGT) by using commercially available GGT estimation kit in Evolution 201 UV-Visible spectrophotometer by carboxy substrate method, C-reactive protein (CRP) by quantitative turbidimetric immunoassay method using commercially available Quantica®CRP kit total protein by using commercially available total protein estimation kit in Evolution 201 UV-Visible spectrophotometer by biuret method and serum cortisol was estimated by using commercially available Canine cortisol ELISA kit as per manufacturer's guidelines.

Statistical analysis was carried out by Statistical Package for the Social Sciences (SPSS) version 20.0. One way analysis of variance (ANOVA) was used for comparing different groups at different time intervals and paired sample t-test was used to compare different time intervals in the same group. Results were presented as mean±standard deviation (mean±SD) and differences were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

Haematological parameters: The mean±SD values of haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) for all the three groups were recorded and depicted in the Table 1.

There was no significant ($P>0.05$) difference noticed in the mean values of Hb, PCV and TEC between different groups at different time intervals throughout the study period. The mean Hb, PCV and TEC values were significantly ($P<0.05$) lower at 30 min, 1 h, 2 h and 3 h intervals after analgesia as compared to 0 min (baseline) value in all the three groups. Decrease in mean Hb, PCV and TEC values could be because of haemodilution as a result of fluid sequestration from tissues to blood after blood loss or due to fluid retention in response to antidiuretic hormone (ADH) secretion (Ali 2018). It is also may be due to fluid administration during peri-operative period (Ali 2018). The decrease in Hb, PCV and TEC values might be due to fluid retention and reluctant haemodilution due to the action of ADH and aldosterone in response to pituitary-adrenal axis stimulation because of pain, and resultant increased sodium ion and decreased potassium ion concentration. Nevertheless, some response might be because of minor blood loss and fluid administration during surgery (Ali 2018). Similar decrease in Hb and PCV was also observed by Bayan *et al.* (2002 b), Sharma *et al.* (2017) and Shinde *et al.* (2018). Significant ($P<0.05$) decrease in TEC after ovariohysterectomy in dogs was reported by Millis *et al.* (1992). The mean Hb, PCV and TEC values were significantly ($P<0.05$) decreased at 1 h interval as compared to 30 min value in all the three groups. The decrease in these values in the present study might be due to the splenic pooling of erythrocytes that occur with most of the anaesthetics or due to haemodilution in response to fluid therapy during continuous rate infusion (CRI) (Kumar *et al.* 2014, Thejasree *et al.* 2018).

The results of group-T are in agreement with the observation of Millis *et al.* (1992), Monteiro *et al.* (2009), and Giorgi *et al.* (2009). However, de Sousa *et al.* (2008) and Brondani *et al.* (2009) have reported non-significant ($P>0.05$) changes in Hb, PCV and TEC following tramadol

Table 1. Mean±SD values of haematological parameters at different time intervals in Group T, P and M

Parameter	Group	0 min	10 min	30 min	1 h	2 h	3 h
Haemoglobin (g/dL)	T	13.96±0.92^{Aa}	14.01±0.79^{Aa}	12.38±0.42^{Ab}	11.03±0.55^{Ac}	10.98±0.76^{Ac}	10.95±0.95^{Ac}
	P	13.88±0.81^{Aa}	13.87±0.84^{Aa}	12.24±0.43^{Ab}	11.18±0.83^{Ac}	11.15±0.77^{Ac}	11.13±0.84^{Ac}
	M	13.61±0.48^{Aa}	13.55±0.47^{Aa}	12.40±0.70^{Ab}	11.28±0.61^{Ac}	11.23±0.25^{Ac}	11.17±0.27^{Ac}
PCV (%)	T	45.65±2.47^{Aa}	45.83±2.89^{Aa}	41.61±1.90^{Ab}	37.65±2.31^{Ac}	37.41±2.69^{Ac}	37.24±3.41^{Ac}
	P	44.55±4.50^{Aa}	43.51±4.45^{Aa}	40.35±2.62^{Ab}	36.96±3.18^{Ac}	36.70±3.26^{Ac}	36.18±3.41^{Ac}
	M	43.85±3.66^{Aa}	43.90±3.91^{Aa}	41.58±3.67^{Ab}	37.05±3.09^{Ac}	36.75±1.52^{Ac}	35.36±1.92^{Ac}
TEC (millions/cu.mm)	T	6.97±0.77^{Aa}	6.84±0.78^{Aa}	5.82±0.53^{Ab}	5.16±0.44^{Ac}	5.04±0.52^{Ac}	5.01±0.49^{Ac}
	P	7.09±0.80^{Aa}	6.93±0.68^{Aa}	5.89±0.54^{Ab}	5.25±0.48^{Ac}	5.12±0.63^{Ac}	5.07±0.55^{Ac}
	M	7.00±0.87^{Aa}	6.97±0.67^{Aa}	5.75±0.82^{Ab}	5.37±0.33^{Ac}	5.21±0.46^{Ac}	5.10±0.47^{Ac}
TLC (thousands/cu.mm)	T	12.31±0.28^{Aa}	12.25±0.25^{Aa}	11.66±0.24^{Aa}	12.19±0.30^{Aa}	12.31±0.31^{Aa}	12.69±0.27^{Aa}
	P	12.73±0.62^{Aa}	12.69±0.52^{Aa}	12.02±0.49^{Aa}	12.46±0.45^{Aa}	12.95±0.51^{ABa}	12.97±0.54^{Aa}
	M	11.70±1.55^{Aa}	11.68±1.56^{Aa}	11.45±1.51^{Aa}	12.05±1.47^{Aa}	13.86±1.30^{Bb}	13.95±1.08^{Bb}
Granulocyte count (%)	T	77.74±1.40^{Aa}	77.89±1.36^{Aa}	78.25±0.99^{Aa}	78.46±1.13^{Aa}	78.63±0.88^{Aa}	79.75±0.62^{Aa}
	P	76.58±0.55^{Aa}	76.86±0.96^{Aa}	77.85±1.08^{Aa}	78.13±1.09^{Aa}	78.33±0.99^{Aa}	78.58±0.77^{Aa}
	M	78.86±1.45^{Aa}	78.92±1.36^{Aa}	79.21±1.27^{Aa}	79.18±1.66^{Aa}	79.35±1.71^{Aa}	80.53±1.74^{Aa}
Lymphocyte count (%)	T	18.05±1.54^{Aa}	17.86±1.48^{Aa}	17.57±1.25^{Aa}	17.51±1.44^{Aa}	17.45±1.25^{Aa}	16.39±1.00^{Aa}
	P	19.06±0.83^{Aa}	18.91±0.90^{Aa}	18.00±1.14^{Aa}	17.89±1.10^{Aa}	17.86±1.02^{Aa}	17.72±0.78^{Aa}
	M	16.64±1.51^{Aa}	16.77±1.41^{Aa}	16.53±1.39^{Aa}	16.69±1.69^{Aa}	16.63±1.72^{Aa}	15.56±1.72^{Aa}
Monocyte count (%)	T	4.21±0.20^{Aa}	4.25±0.28^{Aa}	4.18±0.64^{Aa}	4.03±0.53^{Aa}	3.92±0.51^{Aa}	3.86±0.77^{Aa}
	P	4.36±0.38^{Aa}	4.23±0.40^{Aa}	4.15±0.12^{Aa}	3.98±0.11^{Aa}	3.81±0.11^{Aa}	3.70±0.06^{Aa}
	M	4.50±0.30^{Aa}	4.31±0.30^{Aa}	4.26±0.27^{Aa}	4.13±0.25^{Aa}	4.02±0.25^{Aa}	3.91±0.27^{Aa}

Superscripts A, B between the groups and superscripts a, b, c between the time intervals within a group differ significantly ($P<0.05$).

administration. The results of group-P are in agreement with the findings of earlier workers (Pandey and Sharma 1986, Gill *et al.* 1996, Chandrashekarappa *et al.* 2009). The results of group-M are in agreement with the findings of earlier workers (Dharmaceelan *et al.* 2018).

Total leucocyte count: The mean±SD values of total leucocyte count for all the three groups were recorded and depicted in the Table 1.

A non-significant ($P>0.05$) decrease in mean TLC value up to 30 min after analgesia and thereafter a non-significant ($P>0.05$) increase till the end of study period was noticed in group-T and group-P. In group-M, significant ($P<0.05$) increase in mean TLC was noticed at 2 h interval after analgesia as compared to 0 min (baseline) value. Decrease in mean TLC at 30 min after analgesia might be due to splenic sequestration during anaesthesia. Increase in mean TLC from 1 h interval onwards could be due to post-surgical trauma. Increase in mean TLC in all the groups could be due to addition of leucocyte to the circulation from the marginal pool of leukocyte. It is also attributed to the release of corticosteroids to overcome stress associated with pain. TLC is the indicator of infection, toxins, trauma as well as haemorrhage. The neutrophilic leukocytosis with some shift to left is a sequel of trauma and tissue damage (Ali 2018). The mean TLC was significantly ($P<0.05$) higher in group-M at 2 h interval after analgesia as compared to group-T but no such finding was noticed with respect to group-P. The mean TLC was significantly ($P<0.05$) higher in group-M at 3 h interval after analgesia as compared to group-T and group-P. Ali (2018) observed early decrease in TLC in tramadol group as compared to meloxicam.

Differential leucocyte count: The mean±SD values of differential leucocyte count for all the three groups were recorded and depicted in the Table 1.

There was no noticeable significant ($P>0.05$) difference in the mean values of granulocyte count, lymphocyte count and monocyte count between different groups at different time intervals throughout the study period. The mean granulocyte count was increased non-significantly ($P>0.05$) whereas mean lymphocyte and monocyte counts were decreased non-significantly ($P>0.05$) throughout the study in all the three groups. Increase in neutrophil count and decrease in lymphocyte and monocyte counts in propofol anaesthesia was recorded by David (1993), Kelawala *et al.* (1996), Surbhi (2008) and Ali (2018). A non-significant ($P>0.05$) increase in the number of granulocytes and the subsequent reduction in the number of lymphocytes may be due to extreme stress on the animal during ovariohysterectomy along with aesthetic stress leading to stimulation of the adrenal cortex and subsequent pro-induction of glucocorticoids that act on the circulating neutrophils (Chandrashekarappa *et al.* 2009, Sahoo 2015, Paul 2019). Increase in neutrophil count and a corresponding decrease in lymphocyte count may be associated with initial excitement due to handling of animals and slight pain at the incision site (Sahoo 2015).

Table 2. Mean±SD values of biochemical parameters at different time intervals in Group T, P and M

Parameter	Group	0 min	10 min	30 min	1 h	2 h	3 h
Serum glucose (mg/dL)	T	90.67 ± 7.24^{Aa}	93.50 ± 4.69^{Aa}	114.67 ± 7.93^{Ab}	136.33 ± 6.16^{Ac}	133.83 ± 5.63^{Ac}	122.83 ± 7.54^{Ad}
	P	95.00 ± 5.97^{Aa}	97.17 ± 6.58^{Aa}	113.83 ± 6.73^{Ab}	140.83 ± 4.15^{Ac}	138.50 ± 6.72^{Ac}	125.67 ± 9.77^{Ad}
	M	91.33 ± 6.62^{Aa}	93.67 ± 6.21^{Aa}	120.00 ± 7.51^{Ab}	162.33 ± 6.66^{Bc}	160.50 ± 7.03^{Bc}	158.33 ± 8.28^{Bc}
GGT (U/L)	T	5.21 ± 0.13^{Aa}	5.23 ± 0.22^{Aa}	6.13 ± 0.24^{Ab}	6.08 ± 0.23^{Ab}	5.65 ± 0.24^{Aab}	5.59 ± 0.22^{Aab}
	P	5.15 ± 0.09^{Aa}	5.18 ± 0.05^{Aa}	6.09 ± 0.08^{Ab}	5.94 ± 0.11^{Ab}	5.57 ± 0.12^{Aab}	5.48 ± 0.12^{Aab}
	M	5.23 ± 0.10^{Aa}	5.21 ± 0.09^{Aa}	6.19 ± 0.08^{Ab}	6.11 ± 0.15^{Ab}	5.61 ± 0.15^{Aab}	5.51 ± 0.16^{Aab}
CRP (µg/mL)	T	4.10 ± 0.19^{Aa}	4.14 ± 0.20^{Aa}	4.36 ± 0.16^{Aab}	4.65 ± 0.20^{Ab}	5.05 ± 0.10^{Ac}	5.56 ± 0.10^{Ad}
	P	3.98 ± 0.26^{Aa}	4.07 ± 0.18^{Aa}	4.32 ± 0.17^{Aab}	4.78 ± 0.10^{Ab}	5.95 ± 0.14^{ABc}	6.86 ± 0.14^{Bd}
	M	4.05 ± 0.22^{Aa}	4.09 ± 0.28^{Aa}	4.42 ± 0.24^{Aab}	4.81 ± 0.13^{Ab}	6.75 ± 0.15^{Bc}	7.78 ± 0.26^{Cd}
Total protein (g/dL)	T	6.49 ± 0.17^{Aa}	6.45 ± 0.17^{Aa}	6.28 ± 0.19^{Aa}	5.70 ± 0.22^{Ab}	5.67 ± 0.19^{Ab}	5.62 ± 0.18^{Ab}
	P	6.53 ± 0.34^{Aa}	6.51 ± 0.37^{Aa}	6.38 ± 0.29^{Aa}	5.76 ± 0.38^{Ab}	5.71 ± 0.32^{Ab}	5.58 ± 0.32^{Ab}
	M	6.52 ± 0.24^{Aa}	6.49 ± 0.23^{Aa}	6.32 ± 0.19^{Aa}	5.87 ± 0.18^{Aab}	5.64 ± 0.22^{Ab}	5.46 ± 0.39^{Ab}
Serum cortisol (µg/dL)	T	2.29 ± 0.20^{Aa}	2.33 ± 0.17^{Aa}	4.34 ± 0.36^{Ab}	6.40 ± 0.26^{Ac}	7.00 ± 0.27^{Ad}	7.17 ± 0.29^{Ad}
	P	2.18 ± 0.14^{Aa}	2.26 ± 0.22^{Aa}	4.58 ± 0.49^{Ab}	6.94 ± 0.30^{ABc}	7.75 ± 0.19^{Bd}	7.83 ± 0.21^{Bd}
	M	2.39 ± 0.22^{Aa}	2.44 ± 0.24^{Aa}	4.89 ± 0.57^{Ab}	7.48 ± 0.39^{Bc}	8.32 ± 0.26^{Cd}	8.43 ± 0.38^{Cd}

Superscripts A, B between the groups and superscripts a, b, c, d between the time intervals within a group differ significantly (P<0.05).

Biochemical parameters

Serum glucose: The mean±SD values of serum glucose for all the three groups were recorded and depicted in the Table 2.

The mean glucose concentration was significantly (P<0.05) higher in group- M at 1 h, 2 h and 3 h intervals as compared to group-T and group-P. A significant (P<0.05) increase in mean glucose concentration was noticed at 30 min and then at 1 h interval after analgesia as compared to 0 min (baseline) value in all the three groups. Increase in the mean concentration of glucose noticed in the present study may be due to cortisol and catecholamine mediated gluconeogenesis along with decreased peripheral use of glucose (Bayan *et al.* 2002b). The hyperglycemia observed in the present study could be due to effect of anaesthetic agent on subcortical pathway which produced stress like conditions with increased release of glucocorticoids (Kelawala *et al.*1991, David 1993) which mobilized glucose from tissue into the circulation and also produced gluconeogenesis which is contributed to hyperglycemia. Anandmay *et al.* (2016) also observed significant (P<0.05) increase in blood glucose with propofol anaesthesia at 1 h post induction.

The results of tramadol group are in agreement with earlier workers de Sousa *et al.* (2008) and Kongara *et al.* (2009) and differed from results of Seddighi *et al*. (2009) and Monteiro *et al.* (2009) who reported that tramadol administration will not affect much the biochemical variables. The findings of pentazocine lactate administration in dogs were in agreement with earlier workers Parihar and Pandey (1988), Jit *et al.* (2003) and Chandrashekarappa *et al.* (2009). The findings of Meloxicam administration in dogs were in agreement with Kaushik (2002). The mean glucose concentration was significantly (P<0.05) higher in group-M at 1 h, 2 h and 3 h intervals as compared to group-T and group-P. With regard to the regulation of beta cell insulin secretion, ATP- sensitive K+ (KATP) channels act as a molecular cellular metabolism sensor and play a critical role in the regulation of beta cell insulin secretion (Muir 2009).

Gamma glutamyl transferase: The mean±SD values of gamma glutamyl transferase for all the three groups were recorded and depicted in the Table 2.

There was non-significant (P>0.05) difference in the mean values of GGT between different groups at different time intervals throughout the study period. The mean concentration of GGT was increased significantly (P<0.05) at 30 min after analgesia in all the three groups as compared to 0 min (baseline) value in all the three groups. A significant (P<0.05) increase in GGT level might be due to strong anaesthetic effect of propofol. These findings are similar to results of Nusory (2011) who reported elevated GGT levels during propofol anaesthesia in dogs. However, Anandmay *et al.* (2016) reported a non-significant (P>0.05) increase in GGT values at 1 h interval as compared to baseline in propofol anaesthesia in dogs. GGT is primarily found in the kidney, liver, and pancreatic cells. Smaller concentrations are found in other tissues. The serum enzyme appears to be derived predominantly from the hepatobiliary system, while renal tissue has the highest GGT level, and GGT activity was increased in all forms of liver disease/liver cell injury (Saikia *et al.* 2016). The transient variations in GGT values in the present study were within normal physiological limits might be indicative of non-toxic/less harmful effect of all the anaesthetic drugs on heapato-billiary system.

C-reactive protein: The mean±SD values of c-reactive protein for all the three groups were recorded and depicted in the Table 2.

The mean concentration of CRP was significantly (P<0.05) higher in group- M at 2 h interval as compared to group-T and group-P. At 3 h interval, there was significant (P<0.05) difference noticed in all the three groups. The mean CRP concentration was significantly (P<0.05) increased from 1 h interval till the end of study period in all the three groups and it may be because of surgical trauma. CRP is an important acute phase protein in dogs with systemic inflammation following surgery, trauma, infection, or neoplasia that shows increasing serum concentrations. CRP is a valuable diagnostic marker of systemic inflammation in dogs and automated assays have been validated for accurate measurements for routine diagnostic purposes. CRP is one of the liver-released acute-phase proteins and the liver's production of CRP is upregulated by the circulation of peripheral cytokines and is a highly sensitive marker of the existence of these cytokines. It has been concurrently recognised as a marker of the severity of tissue injury, increasing proportionally with the degree of inflammation, infection or injury (Hassan *et al.* 1990). CRP could be considered as the fastest reacting positive acute phase proteins (APP) seen in dogs (Ceron *et al.* 2005), which increases in response to infection and tissue injury (Conner *et al*. 1988). Although there are some hypothalamic-pituitary-adrenal effects on acute-phase protein concentrations, CRP is primarily triggered by the release of peripheral cytokines and thus should be less affected by factors such as excitement, fear and handling. Several recent studies have reported significant ($P<0.05$) differences between different types of ovariectomy in terms of the CRP concentrations, with less invasive procedures producing the smallest rises in the CRP (Pepys and Hirschfield 2003). After ovariohysterectomy in dogs, CRP, serum amyloid A (SAA) and haptoglobin (Hp) concentrations offered useful information on the warning inflammatory response to surgical insult and could help to track the postoperative path (Dabrowski *et al.* 2007).

The mean concentration of CRP was significantly ($P<0.05$) higher in group-M at 2 h interval as compared to group-T and group-P. This could be due to rapid anti-inflammatory action of tramadol and pentazocine lactate. Tramadol and pentazocine lactate reduce prostaglandin-E2 concentrations in inflammatory exudates, in addition to its well-recognized stimulation of μ-opioid receptors (Buccellati *et al.* 2000). This anti-inflammatory effect of tramadol and pentazocine lactate could stem from the aforementioned effect on serotonergic and noradrenergic transmission which reduces inflammatory edema in experimental conditions in mice (Bamigbade and Langford 1988). In contradictory, Ali (2018) reported that meloxicam is having better anti-inflammatory effect as compared to tramadol and pentazocine lactate.

Total protein: The mean±SD values of total protein for all the three groups were recorded and depicted in the Table 2.

There was non-significant ($P>0.05$) difference in the mean values of total protein between different groups at different time intervals throughout the study period. The mean concentration of total protein was significantly ($P<0.05$) decreased at 1 h interval after analgesia as compared to 0 min (baseline) value in group-T and group-P whereas in group-M such finding was noticed at 2 h interval. Total protein level usually decreases after extensive surgical procedure due to nitrogen loss because of increased adrenal activity and increased protein turnover. During inflammation, fluid and plasma proteins move into extra vascular tissue inducing edema and contributing to decreased albumin (Ali 2018). Haemorrhage and exudation, with shift of large amounts of interstitial fluid plasma compartment, would contribute to hypoproteinemia (Benjamin 1998).

Serum cortisol: The mean±SD values of serum cortisol for all the three groups were recorded and depicted in the Table 2.

The mean concentration of cortisol was significantly ($P<0.05$) higher in group-M and group-P at 1 h, 2 h and 3 h interval as compared to group-T. The mean concentration of cortisol was significantly ($P<0.05$) increased at 30 min and then at 1 h and 2 h intervals as compared to 0 min (baseline) value in all the three groups. It may be due to the increased activity of the hypothalamic-pituitary-adrenal gland. It may be because of stress related to pain, but also due to fear, cold or even the anaesthesia itself. Tissue injury leads to the activation of nociceptive and inflammatory responses that are often associated with pain, hyperalgesia and behavioural changes (Hansen *et al.* 1997). Post-operative behaviour change is a result of negative physical and emotional interactions such as discomfort, insecurity, unhappiness, hunger, thirst, space, partial social isolation, fear, anxiety, stress and pain (Hekman *et al.* 2012).

In reaction to the adrenocorticotropic hormone (ACTH), released from the pituitary gland, cortisol is secreted by the adrenal glands. It increases transiently in response to stressors such as pain, excitement, fear or anxiety, or more chronically in long-term stress. Cortisol concentration assays have been used as a measure of dog stress and pain (Hansen *et al*. 1997, Devitt *et al*. 2005, Hancock *et al*. 2005, Malm *et al*. 2005) and in cats (Smith *et al*. 1996). Marcovich *et al.* (2001) and Hoglund *et al.* (2015) observed that the assessment of cortisol was helpful for testing intraoperative noxious stimuli in dogs. Agnati *et al.* (1991) and Tecot (2007) also reported similar findings. A number of studies have examined the effect of anaesthesia and surgery on cortisol concentration changes in dogs (Ali and Shepherd 1994). Studies have identified that all forms of surgery increases plasma cortisol concentrations markedly (Murata *et al.* 2004). It has also been reported that cortisol responses are altered by different types of analgesic and surgical techniques, with painful or invasive techniques causing greater increase in the cortisol concentration (Halevy *et al.* 1995). Numerous other factors have also been found to influence canine cortisol levels, such as novel experiences, housing and handling, or analgesia without surgery. Significantly ($P<0.05$) increased plasma cortisol level after ovariohysterectomy in dogs and cats has been reported by Fox *et al.* (1994) and Smith *et al.*(1996), respectively. Laiju *et al.* (2011) also reported a significant increase in plasma cortisol level immediately after open surgical ovariohysterectomy in dogs. At 2 h and 3 h intervals, the mean concentration of cortisol was significantly lower in group-T as compared to group-P and group-M. Similar finding was also reported by Ali (2018). This could be due to rapid action of tramadol.

Based on the above findings, it is concluded that the alterations in haematological parameters caused by tramadol, pentazocine lactate and meloxicam were found to be minimal and within the physiological limits. Tramadol produced less significant rise in C-reactive protein and cortisol concentrations as compared to pentazocine lactate and meloxicam which indicates better pain management. Tramadol was found to be more effective as compared to pentazocine lactate and meloxicam in the management of post-operative pain due to canine ovariohysterectomy.

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