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Research Article

Comparative Analgesic Efficacy for Intraperitoneal Administration of Bupivacaine, Ropivacaine, and Ropivacaine-Tizanidine Combination During Ovariohysterectomy of Cats Suffering from Pyometritis

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Abstract

A prospective clinical trial was conducted on 30 cats suffering from pyometritis to investigate the multimodal analgesic efficacy of ropivacaine-tizanidine (RT) combination through intraperitoneal (IP) administration during ovariohysterectomy. Cats were randomly assigned into three groups whereby group RT received 0.5% ropivacaine (1 mg/kg) and tizanidine (10 µg/kg) (IP), group B received 0.5% bupivacaine (2 mg/kg) (IP), and group R received only 0.5% ropivacaine (1 mg/kg) (IP). It was observed that duration for anesthetic recovery (P<0.0001) and extubation time (P=0.0492) differed significantly for group RT. Body temperature and Pulse remained significantly higher in RT group at 1-, and 2-h postoperative intervals, specifically. Interactive Visual Analogue Scale (IVAS) and UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS) scores were significantly lower for group RT at 6-, 8-, and 12-h time periods. Whereas, Mechanical nociceptive threshold (MNT) measurements differed significantly across 1 (P=0.0037), 4 (P=0.0013), 8 (P=0.0024) and 12-h (P=0.0258) time periods. The need for rescue analgesia and Serum cortisol concentrations, consistently remained significantly lower in group RT (P<0.0001). However, values for ALT, AST, ALP, BUN, and creatinine showed no significant differences among groups at 1-, 8-, and 24-h postoperative periods. The study concluded that the ropivacaine-tizanidine combination effectively reduced pain scores, delayed the need for rescue analgesia, and avoided adverse effects.

Keywords: Bupivacaine, Intraperitoneal instillation, Multimodal analgesia, Ropivacaine, Tizanidine, UNESP-Botucatu multidimensional composite pain scale

Introduction

Recent studies highlight the effectiveness of various multimodal analgesic strategies for mitigating postoperative pain and swelling in cats [1]. Among these approaches, intraperitoneal (IP) instillation of local anesthetic agents, emerges as a method capable of effectively managing postoperative pain over an extended duration, reducing the necessity for subsequent administration of anti-inflammatory drugs following major surgical procedures [2,3]. Previous research has established the efficacy of both bupivacaine and ropivacaine in providing prolonged postoperative pain relief with a minimal risk of systemic toxicity [2,4]. Additionally, investigations have compared the cardiotoxic and neurotoxic effects associated with intraperitoneal instillation of these agents as well [5,6]. A preliminary study suggests, that a multimodal approach involving intraperitoneal infusion of local anesthetic agents, could potentially prolong the effects of these drugs [2]. Achieving this would necessitate the concurrent use of α2-adrenergic agonists with these local anesthetics to not only block sensory receptors but also interrupt sensory reflexes

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at the dorsal horn of the spinal column [1]. Tizanidine has been proposed as a suitable drug for directly inhibiting the cation current at the reflex arc level, significantly enhancing the duration and quality of analgesia [7]. The additional effect of tizanidine is speculated to induce localized vasoconstriction, thereby delaying the absorption of local anesthetics into the bloodstream [8]. The longer these agents remain in a circumscribed area, the more prolonged their effective duration of action would be [7]. Despite serious risks associated with systemic tizanidine administration, combining it with other medications or therapies at lower doses could enhance pain control for cats recovering from surgery or managing chronic conditions [9]. Unlike rats, pharmacodynamics of intraperitoneal tizanidine administration in cats has not been extensively researched. However, its efficacy in multimodal analgesic approaches when administered in lower doses has been suggested previously [10].

Recently it has been reported that intraperitoneal instillation of ropivacaine is not as efficacious as that of bupivacaine [11]. However, bupivacaine has been linked to greater instances of systemic toxicity, seizures, cardiac arrhythmias, and respiratory depression when administered intraperitoneally compared to ropivacaine [11,12]. Studies reveal that racemic compounds, such as Bupivacaine, exhibit higher absorptivity but may entail more adverse effects on canine or feline patients compared to the S (-) isomer, ropivacaine [2]. This discrepancy is attributed to the relatively lower lipophilicity of ropivacaine, resulting in milder effects on cardiac function [10]. However, this same characteristic makes ropivacaine less effective in blocking nerve impulses compared to bupivacaine [1]. Consequently, an intervention involving lower doses of tizanidine, in conjunction with ropivacaine could precisely enhance peri-operative analgesia in severely pyometric animals. This is particularly crucial given the significant abdominal distension caused by engorged uterine horns, leading to substantial regional discomfort post-excision [13]. Tizanidine was selected for its unique mechanism of action, perceived systemic safety, potential effectiveness, and its role in a multimodal pain management strategy, all with a primary focus on the well-being of feline patients. This study aimed to assess and compare the postoperative analgesic effects and potential adverse events resulting from the intraperitoneal instillation of bupivacaine, ropivacaine, and a combination of ropivacaine-tizanidine in pyometric cats undergoing ovariohysterectomy. The hypothesis posited by the authors was that the inclusion of tizanidine alongside ropivacaine would significantly enhance postoperative pain relief and prolong the time before rescue analgesia was required, all without adversely affecting liver and kidney functionality.

Material and Methods

Ethical Consideration

Only clinical patients requiring ovariohysterectomy for clinical imperatives were inducted into the study following provision of an informed consent from their respective caretakers. The design of this study followed guidelines outlined by the "Guide for the Care and Use of Laboratory Animals in Research and Teaching". Experiment was conducted under the auspices prescribed by the Ethical Review Committee of the Office of Research, Innovation & Commercialization (ORIC), Cholistan University of Veterinary and Animal Sciences, Bahawalpur-Pakistan (Approval no: ORIC 224) and was legally compliant with Punjab Wildlife Protection, Preservation, Conservation and Management Act (1974).

Selection of Animals

This research, conducted with informed consent from the owners, involved 30 domestic cats of various breeds diagnosed with pyometritis and deemed suitable for ovariohysterectomy for their survival. Preoperative abdominal ultrasonography was performed to exclude pregnancy and confirm pyometritis. The selected cats had an average age and body weight of 16.83±2.92 months and 2.59±0.13 kilograms, respectively. Cats were fasted overnight, and any severely emaciated or unwell patients were excluded. Pregnant, lactating, and fractious animals were also excluded from the study. All cats enrolled in the study were admitted at least 6 h before surgery.

Surgical Groups

Thirty cats inducted into this study were randomly allocated into three experimental groups. Cats in the first group were instilled with a combination of 0.5% ropivacaine (Ropicain; Howards®) at 1 mg/kg and tizanidine (Movax; Sami Pharmaceuticals, Pakistan) at 10 µg/kg (group RT, n = 10) intraperitoneally. A second group of individuals received 0.5% bupivacaine (Bupicain; Lahore Chemical & Pharmaceutical, Pakistan®) at 2 mg/kg (group B, n = 10) intraperitoneally, while a third group was administered with 0.5% ropivacaine (Ropicain; Howards®) at 1 mg/kg (group R, n = 10) during ovariohysterectomy of pyometric animals. Ten 2 mg tizanidine tablets were dissolved in 100 mL of sterile saline, resulting in a 0.02% solution. This solution was administered intraperitoneally at a dose rate of 10 µg/kg, with each drop of the solution roughly measuring 0.05 mL. The number of drops was calculated based on each patient's individual body weight and then added to the mixture to be instilled. For consistency, all solutions containing 0.5% bupivacaine, 0.5% ropivacaine, or a combination of ropivacaine and tizanidine were uniformly reconstituted with normal saline, resulting in a final volume of 1 mL. These preparations were

administered intraperitoneally, succinctly following the placement of ligatures around ovarian pedicle and uterine stump [14]. Considering the ethical implications, to avoid any unfortunate intra-operative pain, meloxicam at 0.03 mg/kg was administered to all cats before anesthetic induction [2].

Anesthetic and Surgical Procedures

The same anesthetist performed all the procedures who was blinded to the animal grouping. A 24-gauge intravenous catheter was aseptically placed in cephalic vein of all the presented patients. An adequate dose of propofol at 6 mg/kg (Diprivan; ICI, Pakistan®) was administered intravenously (IV) to induce sedation and allow for the placement of an endotracheal tube [6]. The endotracheal tube was attached to a non-rebreathing Bain system and isoflurane (Forane, Baxter Healthcare Corporation®) was maintained at a 1.58% Minimum alveolar concentration (MAC) value. Lactated Ringer's solution (Unisol-RL, UNISA Pharmaceutics, Pakistan®) was administered intravenously at 10 mL/kg/h until extubating. Electrocardiography, heart rate, respiratory rate, pulse oximetry, and plethysmography were repeatedly observed during anesthetic duration by employing a multiparametric monitor. A sphygmomanometer with cuff size number 1 was placed at the animal's antebrachium to monitor systolic arterial pressure (SAP). MAC value was adjusted based on the values of physiological parameters (body temperature, pulse rate, breathing rate, systolic arterial blood pressure) and anesthetic reflexes (medio-ventral rotation of the eyeball, loss of jaw tone and palpebral reflexes). Ovariohysterectomy was performed employing a typical technique whereby surgical approach was made through a midline laparotomy incision in dorsally recumbent cats. Same surgeon performed all procedures to alleviate any discrepancies based on the differences in skill and experience. The anesthetic duration was determined by calculating the time elapsed since the induction by propofol till the discontinuation of isoflurane. Whereas the time it took for the surgeon to give primary incision up until the placement of final sutures indicated the duration of surgery. As animals started to recover and a swallowing reflex was observed, they were extubated and time elapsed since termination of isoflurane was noted as well. While overall recovery time was estimated when patients voluntarily resumed their sternal positions.

Scoring for Pain

Trained observers, blinded to the treatment groups and experienced in pain assessment, were designated to assign pain scores. The postoperative impact of drug combinations on animal consciousness and responsiveness was evaluated using an Interactive Visual Analogue Scale (IVAS). In this scale, an elevated pain sensation was indicated by a higher numerical figure on the 0-100 equidistant lines. Additionally, a more comprehensive UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS) ranging from 0 (no pain) to 30 (maximum pain) was employed to subjectively assess the effectiveness of analgesic regimens. Baseline values were established 6 h before surgery, and subsequent readings were recorded at 1, 2, 4, 6, 8, and 12 h post-extubation for both scales. Animals were coerced to move about, inside the cage, once they had recovered from anesthesia. The peri incisional area was palpated and mechanical nociceptive thresholds (MNT) was assessed using homemade von Frey filaments as described by de Sousa et al.[15]. Probing was performed intermittently (6 h pre-op, 1 h, 4 h, 8 h, and 12 h) using Nylon filaments around peri-incisional region, exerting incremental levels of force (0.5, 2.0, 20.0, 39.0, 78.0, 98.0 mN).

Rescue Analgesia and Other Adverse events

Rescue analgesia was accomplished using buprenorphine at 0.01 mg/kg when MCPS was ≥6, whereas other adverse events namely vomiting, diarrhea, seizures, and cardiovascular incidences were recorded as well. Individuals requiring rescue analgesia or that experienced any complications were recorded and subsequently removed from the study as no further observations were made for them.

Laboratory Testing for Blood Glucose and Serum Cortisol Concentrations

Blood glucose and Serum cortisol concentrations could prove useful as biochemical indicators of inflammation and pain. 6 h before surgery and then at 1, 8, and 24 h post-operatively, blood samples were collected for the evaluation of blood glucose and serum cortisol concentrations. Blood glucose concentrations were estimated in mg/dL using a glucometer while collected serum was shipped to a laboratory for serum cortisol analysis using solid phase radioimmunoassay.

Biochemical Testing for Liver and Kidney Function

Blood samples were obtained at various time points i.e., 6 h before surgery and at 1, 8, and 24 h following the procedure. These samples were allowed to coagulate and were then subjected to centrifugation at 3000 rpm for 15 min to separate the serum. The resulting serum was meticulously preserved at -20°C, awaiting subsequent biochemical analysis. Feline liver function was assessed by estimating Alanine aminotransferase (ALT (U/L)), Aspartate transaminase (AST (U/L)), and Alkaline phosphatase (ALP (U/L)) [16]. Whereas, Blood urea nitrogen (BUN (mg/dL)) and Creatinine (mg/dl) were investigated for ascertaining kidney function (Vet Scan VS2 analyzer, ABAXIS®, USA).

Statistical Analysis

Values pertaining to parametric variables such as age, weight, physiological parameters, duration of anesthesia, duration of surgery, extubation time, recovery times, serum cortisol and plasma glucose concentrations were presented as mean ± standard deviation and compared among groups using one-way analysis of variance followed by a Bonferroni test. Whereas nonparametric variables namely UNESP-Botucatu multidimensional composite pain scale scores and mechanical nociceptive threshold values were analyzed using a Kruskal-Wallis's test. Fisher's exact probability test was used to compare number of cats requiring rescue analgesia. Biochemical parameters namely AST, ALT, ALP, BUN and creatinine were also compared for experimental groups using a one-way analysis of variance followed by a pairwise comparison by Dunn's test. All analyses were performed using GraphPad Prism (version 8.4.3) and statistical significance was indicated when $P<0.05$.

Results

Demographic parameters namely, age (range: 11-23 months) (P=0.8270) and body weight (range: 2.4-2.8 kg) (P=0.1470) estimated before the initiation of clinical trial, were statistically non-significant between RT, B, and R groups. This was done to verify the randomness of grouping process and ensure elimination of experimental bias during allocation of individuals into different groups.

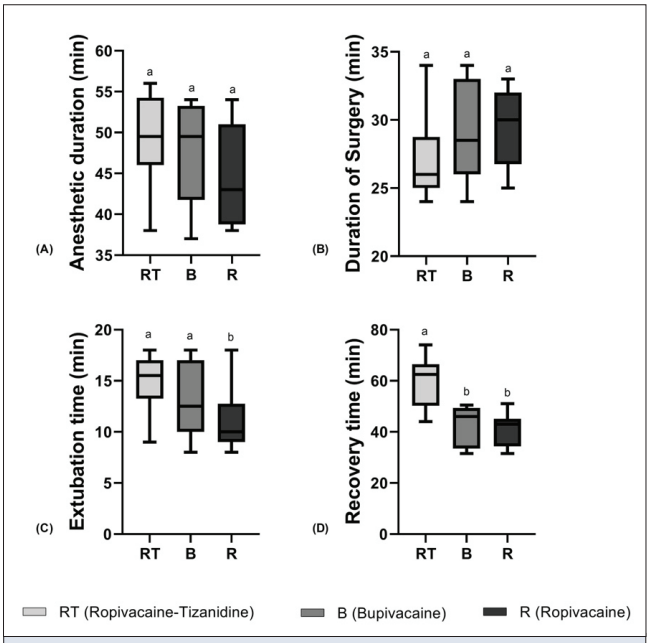

Fig 1. Values for different intraoperative variables represented as Box and Whisker plots in Groups RT, B and R: (A) Average duration of anesthesia (min); (B) Duration of Surgery (min); (C) Extubation time (min); and Recovery time (min) amongst experimental groups (Groups RT, B and R). Whereby significant differences ($P<0.05$) among groups are indicated by different superscripts [(a,b,c)]

Operative durations including surgical (P=0.2547) and anesthetic (P=0.2408) durations were also ensured to be non-significant as a comparable technique was employed by same personnel for all patients. Whereas, duration for anesthetic recovery was significantly different amongst groups when a multiple comparison post-hoc test was performed between ropivacaine-tizanidine group, bupivacaine group (P=0.0001), and ropivacaine group (P=0.2408). Moreover, extubation time was significantly different between group RT and R (P=0.0492) as well *(Fig. 1)*.

All parameters indicating physiological normalcy i.e., body temperature (°F), pulse (beats/min), respiration (breaths/min) and systolic arterial blood pressure (mmHg) were determined 6 h prior to anesthesia to establish base line values for all individuals. No statistical difference was reported amongst any of the groups. Observations were repeated at 1-, 2-, 4-, 6-, 8-, and 12-h intervals postoperatively as well. At 1h postoperatively body temperature was observed to be significantly higher in RT group when compared with B (P=0.0010) and R (P=0.0113). A similar pattern was reported at 2- and 4-h intervals as well, whereby values in RT group remained higher *(Fig. 2)*. While values between groups were non-significant at 6-, 8- and 12-h marks. Pulse rate was observably increased amongst all the study groups relative to their baseline values, but values amongst individuals of RT group were significantly higher at 1 h and 2 h post-operatively whereas with the progression of time this trend changed and by 6th h B group experienced observably higher pulse rates. Respiration rates remained non-significant across all groups throughout the experimental design. While systolic arterial pressure was significantly

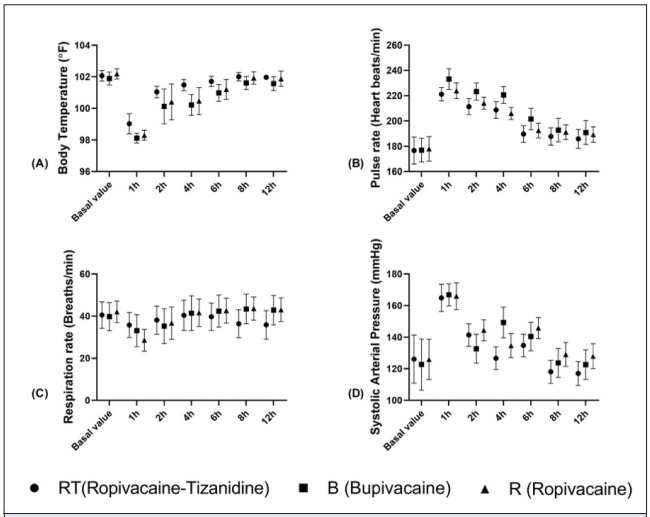

Fig 2. Interleaved symbols graph illustrating Pre- and Post-operative values of: (A) Body temperature (°F); (B) Pulse rate (Heart beats/min); (C) Respiration rate (Breaths/min); and (D) Systolic arterial pressure (mmHg) in surgical patients (Groups RT, B, and R) at Basal value (preoperative assessment 2 h before surgery), 1 h, 2 h, 4 h, 6 h, and 8 h postoperatively. Whereby values are represented as Mean and error bars indicate ±SD

Table 1. *Pain scores for Interactive visual analogue scale (IVAS), Modified Composite Analogue Scale (MCPS) and Mechanical Nociception Test (MNT) represented as median (Range)*

Pain Scales	Groups	Time Interval (hours)						
		Preoperative (6h)	Postoperative					
		Baseline (BL)	1h	2h	4h	6h	8h	12h
IVAS (0-100)	RT (n=10)	0 (0-0) [a]	3.5 (0-15) [a]	5.5 (0-16) [a]	7.5 (0-17) [c]	9.5 (0-22) [c]	14.5 (0-37) [c]	16.5 (12-30) [c]
	B (n=10)	0 (0-0) [a]	7.5 (0-25) [a]	13 (0-26) [a]	17.5 (0-36) [b]	20 (2-30) [b]	28 (3-38) [b]	33 (11-40) [b]
	R (n=10)	0 (0-0) [a]	18.5 (0-30) [a]	19 (0-30) [a]	28.5 (0-38) [a]	29.5 (19-37) [a]	37 (26-40) [a]	44 (31-49) [a]
MCPS (0-30)	RT (n=10)	0 (0-0) [a]	1 (0-2)	1 (0-2)	1 (0-3)	1 (0-3)	3 (1-8)	3 (9-1)
	B (n=10)	0 (0-0) [a]	1.5 (0-4)	2 (0-4)	2 (0-8)	3 (0-5)	7 (2-13)	8 (3-14)
	R (n=10)	0 (0-0) [a]	2 (0-4)	2 (0-5)	3 (0-7)	3 (0-6)	12 (2-16)	15 (14-21)
MNT (mN)	RT (n=10)	98 (98-98) [a]	98 (98-98)	---	98 (78-98)	----	88 (39-98)	88 (39-98)
	B (n=10)	98 (98-98) [a]	88 (78-98)	---	78 (20-98)	---	39 (20-78)	39 (20-78)
	R (n=10)	98 (98-98) [a]	78 (20-98)	---	58.5 (2-98)	---	29.5 (2-39)	20 (2-39)

Group RT (0.5% Ropivacaine at 1 mg/kg and Tizanidine at 10 µg/kg); Group B (0.5% Bupivacaine at 2 mg/kg); Group R (0.5% Ropivacaine at 1 mg/kg). Statistical significance (P<0.05) amongst prospective groups is identified by different superscripts [a,b,c] in a column

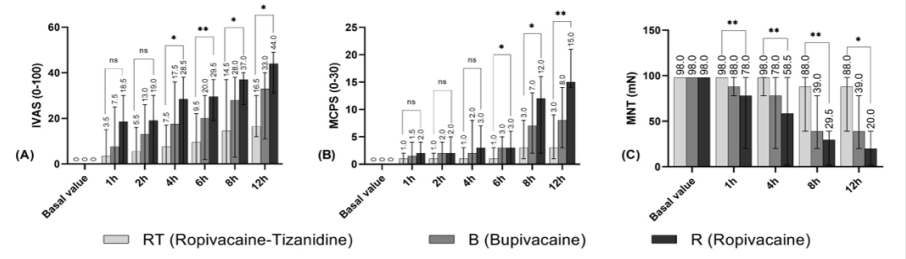

Fig 3. Interleaved bar graphs illustrating mean pain scores: (A) Interactive visual analogue scale (IVAS); (B) Modified Composite Analogue Scale (MCPS); and (C) Mechanical Nociception Test (MNT) represented as median (Range), observed 6 h prior to surgery (baseline) and subsequently at 1 h, 2 h, 4 h, 6 h, 8 h, and 12 h periods postoperatively in 30 cats instilled intraperitoneally with 0.5% Ropivacaine at 1 mg/kg and Tizanidine at 10 µg/kg (group RT, n=10); 0.5% Bupivacaine at 2mg/kg (group B, n=10); or 0.5% Ropivacaine at 1 mg/kg (group R, n=10). Whereby significant differences (P<0.05) among groups are indicated by different superscripts [a,b,c]

higher in group RT as opposed to group B (P<0.0001) at 4-h mark, but continued to remain different from only group R, at subsequent time intervals *(Fig. 2)*.

When compared with baseline IVAS scores were observably higher in subsequent postoperative periods. At 4-, 6-, 8-, and 12-h time intervals the values amongst groups were statistically different from each other whereby P=0.0279, P=0.0014, P=0.0134 and P=0.0138 respectively *(Table 1)*.

A similar trend was observable in the case of MCPS values, whereby significant difference was estimated at 6 (P=0.0193), 8 (P=0.0457) and 12 h (P=0.0011) *(Fig. 3)*. However, the MNT measurements differed significantly across 1 (P=0.0037), 4 (P=0.0013), 8 (P=0.0024) and 12-h (P=0.0258) time periods *(Fig. 3)*.

Number of cats requiring rescue analgesia were significantly lower for group RT as opposed to other treatment groups (P<0.0001). In case of group B, rescue analgesia was administered to 1, 5 and 4 cats at 4-h, 8-h and 12-h respectively. While 2 cats at 4-h, 1 cat at 6-h, 4 cats at 8-h, 3 cats at 12-h mark had to be rescued in case of group R. No serious complications or other adverse events were observed during the period of this study. Elevated serum cortisol levels were reported for all groups post-operatively, however, greatest cortisol values were observed in patients of group R while the lowest were seen for group RT (P<0.0001) at 1 h mark postoperatively *(Fig. 4)*. The numerical differences continued to decrease by 8 h and 24 h periods. Blood glucose concentrations were statistically significant across all time periods between different groups (P<0.0001) *(Fig. 4)*.

Biochemical parameters namely, alanine aminotransferase (ALT (IU/L)), aspartate transaminase (AST (IU/L)), alkaline phosphatase (ALP (IU/L)), blood urea nitrogen (BUN (mg/dL)) and creatinine (mg/dL) were compared for experimental groups using a one-way analysis of variance *(Fig. 5)*.

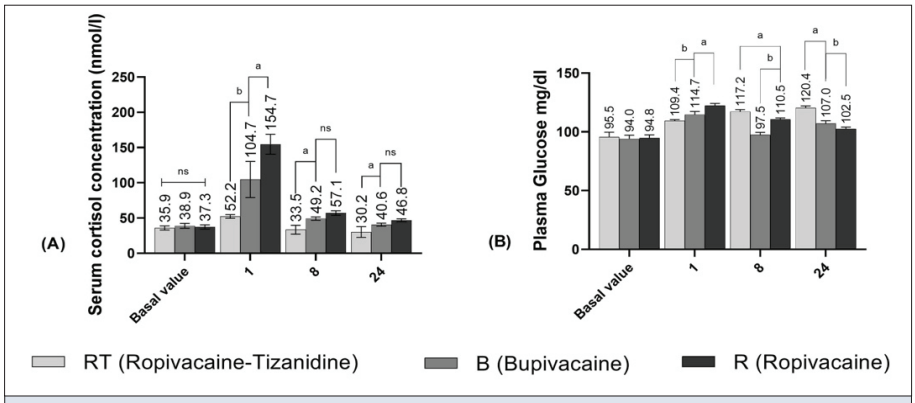

Fig 4. Interleaved bars illustrating: (A) Serum cortisol concentrations (nmol/L); and (B) Plasma Glucose concentration (mg/dL) as (Mean ± SD) values, observed 6 h prior to surgery (baseline) and subsequently at 1 h, 8 h, and 24 h periods postoperatively in 30 cats instilled intraperitoneally with 0.5% Ropivacaine at 1 mg/kg and Tizanidine at 10 µg/kg (group RT, n=10); 0.5% Bupivacaine at 2 mg/kg (group B, n = 10); or 0.5% Ropivacaine at 1 mg/kg (group R, n=10). Whereby significant differences (P<0.05) among groups are indicated by different superscripts (a,b,c)

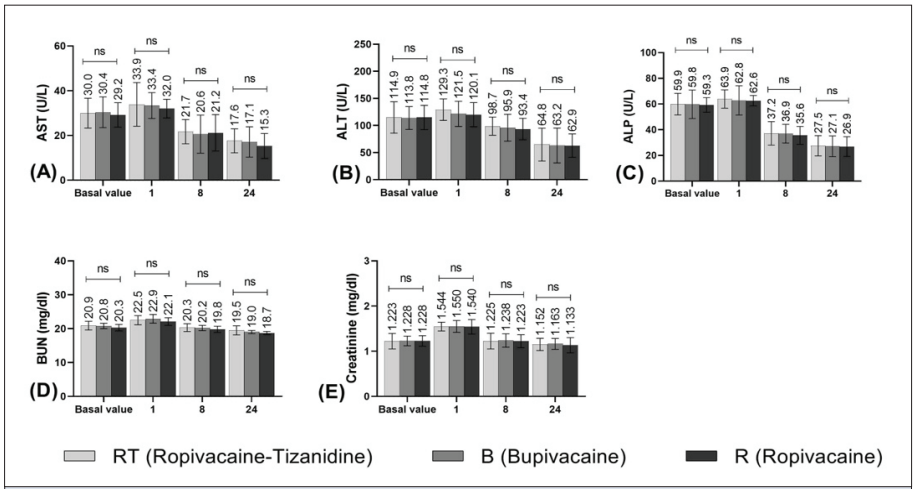

Fig 5. Interleaved bars illustrating: (A) Aspartate transaminase (AST (U/L)); (B) Alanine aminotransferase (ALT (U/L)); (C) Alkaline phosphatase (ALP (U/L)); (D) Blood urea nitrogen (BUN (mg/dL)) and (E) Creatinine (mg/dL) as (Mean ± SD) values, observed 6 h prior to surgery (baseline) and subsequently at 1 h, 8 h, and 24 h periods postoperatively in 30 cats instilled intraperitoneally with 0.5% Ropivacaine at 1 mg/kg and Tizanidine at 10 µg/kg (group RT, n=10); 0.5% Bupivacaine at 2 mg/kg (group B, n=10); or 0.5% Ropivacaine at 1 mg/kg (group R, n=10). Whereby significant differences (P<0.05) among groups are indicated by different superscripts (a,b,c)

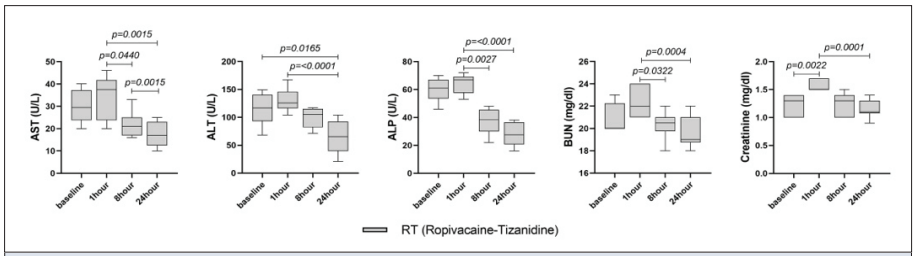

Fig 6. Box and whisker plot illustrating pair wise analysis of group RT (Ropivacaine-Tizanidine) using Dunn's multiple comparisons test for biochemical parameters namely Aspartate transaminase (AST (U/L), Alanine aminotransferase (ALT (U/L), Alkaline phosphatase (ALP (U/L), Blood urea nitrogen (BUN (mg/dL)) and Creatinine (mg/dL) observed 6 h prior to surgery (baseline) and subsequently at 1 h, 8 h, and 24 h periods postoperatively in 30 cats

A post-hoc Bonferroni test was also performed for multiple comparisons amongst groups. None of the variables were statistically different for Group RT, B and R, at baseline, 1, 8 or 24 h of the experimental period *(Table 2)*.

Pairwise analyses within groups were conducted using Dunn's multiple comparison test. In the RT, B, and R groups, all parameters, including ALT, AST, ALP, BUN, and creatinine, exhibited statistically significant differences

Table 2. *Biochemical Parameters for Liver and Kidney function represented as mean ± SD*

Biochemical Parameters for Liver and Kidney Function	Groups	Time Interval (hours)			
		Preoperative (6h)	Postoperative		
		Baseline (BL)	1h	8h	24h
Aspartate transaminase (AST, U/L)	RT (n=10)	30±6.7 ^{aA}	33.9±9.8^{aA}	21.7±5.4 ^{aB}	17.6±5.4 ^{aB}
	B (n=10))	30.4±6.88 ^{aA}	33.4±5.8 ^{aA}	20.6±8.5 ^{aB}	17.1±6.8 ^{aB}
	R (n=10)	29.2±5.45 ^{aA}	32±4.19 ^{aA}	21.2±8.1 ^{aB}	15.3±5.6 ^{aB}
Alanine aminotransferase (ALT, U/L)	RT (n=10)	114.9±29 ^{aA}	129.3±19 ^{aA}	98.7±17 ^{aA}	64.8±30 ^{aB}
	B (n=10)	113.8±21 ^{aA}	121.5±23 ^{aA}	95.9±25 ^{aA}	63.2±32 ^{aB}
	R (n=10)	114.8±22 ^{aA}	120.1±22 ^{aA}	93.4±20 ^{aA}	62.9±22 ^{aB}
Alkaline phosphatase (ALP, U/L)	RT (n=10)	59.9±8.4 ^{aA}	63.9±7.1 ^{aA}	37.2±9.1 ^{aB}	27.5±7.9 ^{aB}
	B (n=10)	59.8±11.1 ^{aA}	62.8±11.4 ^{aA}	36.9±7.3 ^{aB}	27.1±8.1 ^{aB}
	R (n=10)	59.3±5.64 ^{aA}	62.6±4 ^{aA}	35.6±7 ^{aB}	26.9±7.6 ^{aB}
Blood urea nitrogen (BUN, mg/dL)	RT (n=10)	20.9±1.3 ^{aA}	22.5±1.4 ^{aA}	20.3±1.2 ^{aA}	19.5±1.4 ^{aB}
	B (n=10)	20.8±0.79 ^{aA}	22.9±1.3 ^{aA}	20.2±0.8 ^{aB}	19±0.5 ^{aB}
	R (n=10)	20.3±0.95 ^{aA}	22.1±1.1 ^{aA}	19.8±0.9 ^{aB}	18.7±0.5 ^{aB}
Creatinine (mg/dL)	RT (n=10)	1.22±0.17 ^{aA}	1.54±0.1 ^{aB}	1.23±0.2 ^{aC}	1.15±0.1 ^{aC}
	B (n=10)	1.23±0.11 ^{aA}	1.55±0.3 ^{aB}	1.24±0.2 ^{aC}	1.16±0.1 ^{aC}
	R (n=10)	1.23±0.12 ^{aA}	1.54±0.16 ^{aB}	1.22±0.14 ^{aC}	1.13±0.17 ^{aC}

Group RT (0.5% Ropivacaine at 1 mg/kg and Tizanidine at 10 µg/kg); Group B (0.5% Bupivacaine at 2 mg/kg); Group R (0.5% Ropivacaine at 1 mg/kg). Statistical significance (P<0.05) amongst prospective groups is identified by different superscripts $^{(a,b,c)}$ in a column. Whereas, Statistical significance (P<0.05) within groups at different time periods resulting from Dunn's multiple comparison tests, are denoted by different superscripts $^{(A,B\,C)}$ in a row

between baseline and 24 h postoperatively, with baseline values being notably higher *(Table 2)*. In the RT group, AST values showed significant differences between 1 and 8 h (P = 0.0440) and between 8 and 24 h (P=0.0015). Meanwhile, ALT values displayed significant differences at both baseline (P=0.0165) and 1 h (P<0.0001) when compared with the 24-h samples. ALP values followed a similar pattern to AST, with statistical differences observed between 1 and 8 h (P=0.0027) and 1 and 24 h (P<0.0001) *(Fig. 6)*. Both BUN (P=0.0004) and creatinine (P=0.0001) levels were statistically different between 1 and 24 h postoperatively.

Discussion

In our study, combining tizanidine with ropivacaine intraperitoneally during ovariohysterectomy of pyometric cats, significantly improved analgesic efficacy. This approach aimed to prolong the time before rescue analgesia and minimize adverse events associated with instilling bupivacaine or ropivacaine alone. The results of this study corroborated prior findings whereby addition of an alpha 2-agonist to a local instillation protocol was employed to produce a pronounced analgesic event [2]. Previously, when two intraperitoneal (IP) regimens of 0.25% bupivacaine-dexmedetomidine and bupivacaine-epinephrine were compared, researchers observed similar postoperative pain scores. However, they noted a general reduction in the overall frequency of rescue analgesia events for the bupivacaine-dexmedetomidine group, indicating improved efficacy following the addition of an alpha-2 agonist [17]. Similarly, in current settings, authors have observed a positive response in terms of both the postoperative pain scores and rescue analgesic events. A contemporary finding has revealed lower efficacy of Ropivacaine as compared to Bupivacaine in dogs. Out of 22 dogs instilled intraperitoneally with 0.5% bupivacaine, only 6 required rescue analgesia. [11]. Similar outcomes were observed by authors in our study as well, where rescue analgesia was administered to 1, 5 and 4 cats at 4-h, 8-h and 12-h respectively in case of 0.5% bupivacaine while 2 cats at 4-h, 1 cat at 6-h, 4 cats at 8-h, 3 cats at 12-h mark had to be rescued in groups administered with 0.5% bupivacaine. Nevertheless, despite exhibiting identical outcomes, both studies were quite different in terms of experimental design, clinical settings and experimental species. Several other discrepancies observed in our study as opposed to contemporary findings could be rationalized by the inherent differences in experimental approaches [4,17].

Authors employed two different scoring systems to evaluate the post operative analgesic ability of ropivacaine

and tizanidine, namely IVAS and MCPS [2]. Prior investigations have indicated that bupivacaine proved to be a better alternative to ropivacaine when administered intraperitoneally [17]. However, the toxicity and other adverse reactions associated with bupivacaine have deemed it desirable to investigate alternatives. Ropivacaine could prove an alternative but its ability to affect psychomotor functionality is quite limited [12]. Thereby, leading researchers to conduct experiments using various concoctions made with alpha-2 agonists [17]. The assessment of pain in cats in this study focused on two domains, pain expression and psychomotor changes [12]. Due to instrumental limitations, specifically the lack of an electroencephalogram (EEG) for brain wave mapping, the ability to draw conclusions regarding psychomotor changes was restricted. Therefore, the current study relied heavily on physiological norms (temperature, pulse, and respiration) and pain scores for making inferences. The estimation of sedation, depth and degree of consciousness heavily relied on IVAS scores, while composite pain scores served as the primary indicators for evaluating postoperative pain [18]. Consequently, the assessors had to be adequately trained beforehand to properly gauge the degree of pain, considering the highly subjective nature of these scales [19]. In the present study, the relatively low pain scores observed across individuals in all experimental groups could be attributed to comparable study conditions and the expertise of an experienced surgeon who performed all the surgeries with minimal tissue trauma. However, it should be noted that the low pain scores for IVAS and MCPS within the first 4 h after surgery may have made it challenging for the assessors to differentiate among groups, as the overall scores were exceptionally low [1]. On the other hand, objective parameters such as nociception threshold scores (MNT) showed noticeable differences among groups. Overall, these findings highlight the importance of considering both subjective and objective measures when assessing pain in cats and emphasize the need for further investigation in this area [2].

Rescue analgesia for all individuals was managed using a single dose of buprenorphine at 0.01 mg/kg [17,20]. However, prior investigations have also employed short-acting opioids such meperidine for similar purposes [2]. To approximate the experimental design with everyday clinical situations and due to ethical concerns about inefficacy of our intraperitoneal instillations in controlling intraoperative pain, meloxicam was administered to all cats before surgery. Meloxicam has been commonly used for its analgesic properties in cats undergoing various surgical procedures, including ovariohysterectomy (spay) [21]. However, it is important to consider potential effects on clotting when using meloxicam as a premedication [1]. It has also been reported to exert a potent anti-hyperalgesic effect, leading to reduced behavioral responses following ovariohysterectomy [22]. Moreover, local anesthetics, namely bupivacaine and ropivacaine, have the potential to induce cardiac side effects, including rare instances of conduction disturbances, such as AV blocks [6]. Similar to previous studies, authors observed development of AV blocks in 2 of the cats receiving Ropivacaine IP instillation [10]. The incidence of these effects in cats is considered to be very low, however, it's important to note that individual responses to medications can vary, and certain factors such as the dose, concentration, and rate of administration may influence the risk of adverse effects [12]. Additionally, pre-existing cardiac conditions or concurrent administration of other medications may also increase the potential for cardiac complications. Therefore, in our current study both bradycardia and AV block were considered minor complications and did not require specific treatment.

Unlike these amide-type local anesthetics, tizanidine is an alpha-2 agonist notorious for inducing severe side effects in humans [23]. Tizanidine is a muscle relaxant primarily used in humans [7]. A small number of cats have reportedly exhibited hypersensitivity towards tizanidine and other similar drugs from same class [24]. Notably, tizanidine undergoes liver metabolism, making cats with pre-existing liver issues less suitable candidates for its use [24]. However, research has shown that hepatic toxicity is less common in cats than in humans [25]. Some cats may experience nausea, vomiting, diarrhea, and cardiac arrythmias when taking tizanidine, but these issues have been related to the use of all α2-adrenergic class agonists [26]. Most of these concerns were associated with prolonged usage and higher dose rates of at least 25-50 µg/kg [27]. Researchers have postulated that tizanidine could be considered in cats experiencing severe muscle spasms or spasticity due to neurological or musculoskeletal conditions. It could provide relief by reducing excessive muscle contractions [27]. Nevertheless, in the current study, the authors conducted a comprehensive risk-benefit analysis and hypothesized that employing a lower, one-time dose of tizanidine would not only effectively manage the spasticity [7] of abdominal musculature following the removal of an engorged uterus but also alleviate the neuralgia [24] associated with the transection of the ovarian pedicle. Hence, in order to confirm the safety of short-term intraperitoneal administration of tizanidine in cats, both preoperatively (6 h prior) and postoperatively (at 1, 8, and 24 h), serum biochemistry assessments were performed to examine the levels of AST, ALT, ALP, BUN, and creatinine. These biochemical parameters have been considered as effective biomarkers for assessment of hepatic and renal function [28]. In the current study, the authors observed noteworthy deviations in the mean baseline values of AST, ALT, and ALP from the

established reference values [16], while BUN and creatinine exhibited relatively minimal variation. Previous research has indicated that in cases of pyometra, bacteria present in the infected uterus may release harmful toxins into the bloodstream [29], potentially affecting liver function and giving rise to a condition referred to as toxic liver syndrome. Furthermore, substantial losses of electrolytes and fluids through discharge, along with reduced blood flow to the kidneys due to a shared vascular supply, could contribute to the development of acute kidney injury [30]. Authors observed that none of the variables were statistically different for Group RT, B and R, at baseline, 1, 8 or 24 h of the experimental period, indicating that there was no additional deleterious consequence of including tizanidine to local anesthetic concoctions. The subjects enrolled in the current study were confirmed to have pyometritis through clinical observations and ultrasonography. Consequently, it was anticipated that hepatic and renal function might be compromised, and this expectation was substantiated when baseline values were found to be notably higher than the reference range [16]. However, consistent with findings from prior investigations, following the successful completion of ovariohysterectomy, values of AST, ALT, ALP, BUN, and creatinine steadily decreased over time, eventually falling well within the safety parameters [29,30]. This observation underscored that neither the intraperitoneal administration of tizanidine in low doses nor that of amide local anesthetics had a significant impact on the liver and kidney functionality of the patients.

The authors faced a potential constraint regarding the dose rates of tizanidine, given the scarcity of in-depth research on this drug in cats [24]. Additionally, converting tizanidine from tablet form to a solution may have affected its physical and chemical properties, potentially impacting its onset of action, duration, and effectiveness in blocking sensory responses. However, it is important to note that no significant signs of systemic toxicity were observed at the dose and concentration of tizanidine utilized in this study. Tizanidine, being a monoisotopic drug has an absolute oral bioavailability of approximately 40% [31]. About 30% of the drug binds to plasma proteins, and a substantial portion undergoes hepatic metabolism primarily via CYP1A2 [32]. In capsule or tablet formulations, the active ingredient is presented as tizanidine hydrochloride (DS 103-282) [33]. These formulations contain minimal amounts of inactive ingredients like Anhydrous lactose and Microcrystalline cellulose, resulting in a relatively water-soluble form (0.133 mg/mL) [31]. The decision to reconstitute tizanidine tablets in sterile saline was in line with prior research, particularly in rodent models [34-37], where researchers demonstrated the feasibility and effectiveness of intraperitoneal delivery. The rationale for this method was grounded in the higher bioavailability associated with intraperitoneal administration, bypassing the first-pass metabolism in the liver [38]. Although existing literature predominantly discussed such practices in rodent studies, our findings emphasize the necessity for further exploration in feline medicine.

In conclusion, as part of a multimodal pain therapy for cats undergoing ovariohysterectomy, instilling ropivacaine-tizanidine onto the ovarian pedicles and uterine body after excision resulted in significantly pronounced postoperative analgesic effects without exhibiting any pronounced hepatic or renal adverse reactions. However, further investigations may be needed to identify the analgesic efficacy of tizanidine as analgesic treatment in cats.

Availability of Data and Materials

The authors declare that the experimental data supporting the present study findings have been made available to the corresponding author (A.H. Rabbani).

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Conflict of Interest

I hereby declare that there are no conflicts of interest associated with this research. The study is purely academic and aims to contribute to the existing knowledge in the field of veterinary medicine.

Author Contributions

Experimental Design was conceived by AHR, ON and KH. Data was collected by AHR, MS, and QU. Statistical analysis was conducted by ASA and MLS. Original draft was written by AHR and FW. Revision and final proof-reading of the manuscript were accomplished by ON, KH, and FW.

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