Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Studying the local anaesthetic effects of lidocaine, remifentanil (with and without adjuvants), and tramadol in rabbits

Nabaa Fadhil ABBAS *, Raffal A. OMAR

Physiology, Biochemistry & Pharmacology Department, College of Veterinary Medicine, University of Baghdad, Iraq.

* Corresponding Author. E-mail: nabaa.fadel1106h@covm.uobaghdad.edu.iq (N.A.); Tel. +964 773 759 8192.

Received: 4 April 2024 / Revised: 22 April 2024 / Accepted: 23 April 2024

ABSTRACT: Tramadol and remifentanil, which is an opioid analgesic are known to have a local anesthetic effect and the present study aimed to evaluate the local anesthetic efficacy of them on twenty-five adult male rabbits Oryctolagus cuniculus (aged between 10-12 months and weighing 1.5±0.08g) who were divided into five groups received the following treatment subcutaneously for 5 days; G1: 5% Tramadol 15 mg/kg BW; G2: 2% Remifentanil 2 mg/kg BW; G3: 2% Lidocaine 4 mg/kg BW; G4: 2% Remifentanil with epinephrine 0.00l mg/kg BW; and G5:2% Remifentanil with Carbopol gel 2 mg/kg BW. Baseline, pre-anesthetic, and post-anesthetic physiological parameters, including temperature, respiratory rate, and heart rate, were meticulously recorded for all groups following local administration of lidocaine, tramadol, and remifentanil. The results showed that tramadol caused a significant decrease in the temperature while other groups showed non-significant effect, results also revealed that pain evaluation that performed according to Grimace Scale scores and by the behavioral analysis of pain assessment elucidated that Lidocaine treatment group showed a statistically significant lower pain response compared to the Tramadol and Remifentanil groups and further significant improvement in pain were observed with the addition of epinephrine or Carbopol gel to Remifentanil. Both combinations resulted in a statistically significant decrease in pain parameters for all measured features comparing with Tramadol and Remifentanil groups and these results leads to conclude that tramadol and remifentanil showed a comparable anaesthetic effect that improved significantly with epinephrine or carbopol gel as local anaesthetics that may surpass lidocaine local anaesthetic effect significantly.

KEYWORDS: lidocaine; remifentanil; local anesthetics; tramadol; rabbits; adjuvants

1. INTRODUCTION

Local anesthetics are a cornerstone of modern medical practice, providing the essential benefit of temporary sensory loss in targeted areas of the body [1] They are indispensable across a wide array of medical interventions from simple dental work to complex surgical procedures [2]. The fundamental role of these agents is to inhibit nerve impulses, effectively blocking the pain signals before they reach the central nervous system [3]. This mechanism is crucial as it allows medical practitioners to carry out procedures with minimal patient discomfort. Local anesthetics are broadly categorized into two types: esters and amides [4]. Esters, such as procaine and benzocaine, are characterized by rapid metabolism within the body, leading to a shorter duration of action [5]. Amides, conversely, offer a longer anesthetic effect and include agents commonly utilized in clinical settings, like lidocaine, bupivacaine, and ropivacaine [6]. Lidocaine, in particular, is favored for its quick onset, potent anesthetic properties, and low toxicity profile [7]. The pursuit of optimizing the efficacy and safety of local anesthetics has led to numerous studies that compare the pain control efficacy of lidocaine versus bupivacaine in dental procedures which demonstrated that while both were effective, bupivacaine offered an extended duration of analgesia [8, 9].

Pain is a sensory and emotional experience that is influenced by physiological, sensory, affective, cognitive, socio- cultural, and behavioral factors [10]. Lidocaine considered as the most popular local anesthetic as it is safe, rapidly metabolized, and has a short duration of action [11]. Efficacy is enhanced markedly by the addition of adrenaline [12]. A testament to the relative safety of the use of lidocaine is the fact that the drug is used systemically as a class 1b antiarrhythmic and in the treatment of chronic pain that is refractory to alternative approaches [13]. Local anesthetics result in a slight increase in heart rate (HR) due

How to cite this article: Abbas NF, Omar RA. Studying the Local Anaesthetic Effects of Lidocaine, Remifentanil (with and without adjuvants), and Tramadol in Rabbits. J Res Pharm. 2024; 28(4): 1124-1134.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.794

to a specific decrease in arterial pressure [14]. These hemodynamic changes are due to combined effects to the induction drug and use of Laryngeal mask airway [15]. Hemodynamic stability is very important throughout induction of general anesthesia in surgical operations [16]. Thus, anesthetic agent by way of minimum effect on HR and blood pressure [BP] would be the drug of choice for general anesthesia [17], to achieve adequate preoperative, intraoperative, and postoperative anesthesia and analgesia [18] via pain control by using local anesthetics, which are considered safer than general-type anesthetics [19]. The short time onsite is the most important problem for anesthetic action locally [20].

Using anesthetic procedures and agents, local anesthesia has a long history in the field of surgery [21]. Due to advances in knowledge of anesthetic efforts, many diagnostic procedures and a number of surgical operations can be performed under local anesthesia [22]. Local anesthetics agents that reversibly interfere with neural conduction and are widely used to provide pain control in surgical operation, and the ideal local anesthetic solution should be provide best fit for the subjects' systemic condition leading to best surgical interference [23]. Many chemical agents have been developed and produced for local anesthetic purposes, and only a certain number of these substances are currently used [24, 25]. Tramadol is known as a local anesthetic action on peripheral nerves and used to treat acute and chronic pain of moderate to severe intensity to remove painful conditions after recovery from surgery with minimum side effect to reduce risk and cost that associated with general anesthesia in equine practice [26]. The opioid analgesic remifentanil has recently been investigated for its potential as a local anesthetic [27] particularly for short-term analgesic requirements due to its quick onset and cessation of action. This makes remifentanil a candidate for brief surgical procedures and immediate pain relief. Tramadol is primarily known as an opioid pain reliever, but it also exhibits local anesthetic properties by blocking sodium [28]. Some studies have explored its potential as a local anesthetic in dental and minor surgical procedures [29]. The primary application of tramadol continues to be for pain management [30]. For that reason, this study aimed to evaluate the efficacy of tramadol and remifentanil as local anesthetics in rabbits either alone or in combinations with epinephrine ore Carbopol gel which reported previously to provide prolonged duration of action for the anesthetics used in the present work.

2. RESULTS

2.1. Effect of Drug on Temperature

2.1.1. Within-group comparison

Results illustrated in Table 1 showed that for Tramadol group, there was a significant decrease ($p<0.05$) in temperature after injection (from 39.15 ± 0.06°C to 38.27 ± 0.24°C), indicating that the drug had a cooling effect. On the other hand, the Remifentanil group showed a non-significant ($p>0.05$) change in temperature post-injection (39.12 ± 0.06°C to 39.10 ± 0.04°C). The Lidocaine group also showed a non-significant change and rabbits received Remifentanil with epinephrine or carbopol gel also showed a non-significant change in the temperaturs.

2.1.2. Between-group comparison

It was demonstrated in Table 1 that the only group that showed significant changes in the temperature was the tramadol group which showed a significant decrease in temperature after receiving the treatment compared to before injection. Additionally, when comparing the temperatures of all studied groups after-injection, it was demonstrated that tramadol group had a decrease in temperature compared to Remifentanil Lidocaine and remifentanil with carbopol gel group. The Remifentanil with epinephrine group also had a lower temperature compared to the Remifentanil group, but this was not statistically significant. There was no significant difference in the temperature change after injection between the Remifentanil, Lidocaine, and Remifentanil with carbopol gel groups. The purpose of this addition for prolonged duration of action for remifentanil

Table 1. Effect of drug injection on temperature measures in rabbits

Groups/ Drug	Mean ± SE of Temperature	
	Before injection (at 0 time)	After injection
Tramadol	39.15 ±0.06	38.27 ±0.24
	A a	B b
Remifentanil	39.12 ±0.06	39.10 ±0.04
	A a	A a
Lidocaine	39.15 ±0.06	39.12 ±0.07
	A a	A a
Remifentanil with epinephrine	39.15 ±0.06	38.62 ±0.24
	A a	A a
Remifentanil with Carbopol gel	39.07 ±0.04	39.10 ±0.05
	A a	A a
LSD value	0.637 *	

*Means with different big letters in the same column and small letters in the same row are significantly different, * (P≤0.05).

2.2. Effect of Drug Injection on Heart rate

2.2.1.Within-group comparison

The Tramadol group showed a slight increase in heart rate after drug injection. This was not statistically significant. The Remifentanil group experienced a minor decrease in heart rate post-injection, which was also not statistically significant (from 142.33 ± 0.88 bpm to 141.33 ± 0.67 bpm). No significant change in heart rate was observed in the Lidocaine group (143.33 ± 1.76 bpm to 142.33 ± 1.76 bpm,). The Remifentanil with epinephrine group's heart rate remained essentially unchanged after injection (141.00 ± 0.57 bpm to 141.33 ± 0.33 bpm). Similarly, the Remifentanil with carbopol gel group showed no significant change in heart rate (141.33 ± 0.67 bpm to 141.33 ± 0.88 bpm) as illustrated in Table 2

2.2.2. Between-group comparison

There were no statistically significant differences observed between any of the groups in terms of heart rate change post-injection. All groups maintained a similar heart rate, with minor fluctuations that were not significant.

Table 2. Effect of drug ınjection on heart rate measures in rabbits

Groups/ Drug	Mean ± SE of Heart rate	
	Before drug	**After injection drug**
Tramadol	143.00 ±1.52	144.00 ±0.57
	A a	A a
Remifentanil	142.33 ±0.88	141.33 ±0.67
	A a	A a
Lidocaine	143.33 ±1.76	142.33 ±1.76
	A a	A a
Remifentanil with epinephrine	141.00 ±0.57	141.33 ±0.33
	A a	A a
Remifentanil with Carbopol gel	141.33 ±0.67	141.33 ±0.88
	A a	A a
LSD value	**4.029 NS**	

*NS: Non-Significant.

2.3.Effect of Drug Injection on respiratory rate

2.3.1. Within-group comparison

The Tramadol group showed no significant change in respiratory rate after drug injection (from 45.33 ± 3.17 to 45.00 ± 3.00). The Remifentanil group also showed no significant change in respiratory rate (from 38.67 ± 0.33 to 38.33 ± 0.33). No significant change in respiratory rate was observed in the Lidocaine group (from 41.33 ± 3.38 to 41.67 ± 2.73). The Remifentanil with epinephrine group's respiratory rate decreased slightly, but not significantly (from 42.00 ± 3.51 to 38.67 ± 0.33). Similarly, the Remifentanil with carbopol gel group showed no significant change in respiratory rate (from 38.00 ± 1.00 to 38.33 ± 0.33) as shown in Table 3.

2.3.2. Between-group comparison

There were no statistically significant differences observed between any of the groups in terms of respiratory rate change post-injection. All groups showed similar respiratory rates after injection with minor non-significant fluctuations.

Table 3. Effect of Drug Injection on respiratory rate Measures in Rabbits

Groups/ Drug	Mean ± SE of Respiratory rate	
	Before drug	After injection drug
Tramadol	45.33 ±3.17 A a	45.00 ±3.00 A a
Remifentanil	38.67 ±0.33 B a	38.33 ±0.33 B a
Lidocaine	41.33 ±3.38 AB a	41.67 ±2.73 AB a
Remifentanil with epinephrine	42.00 ±3.51 AB a	38.67 ±0.33 B a
Remifentanil with Carbopol gel	38.00 ±1.00 B a	38.33 ±0.33 B a
LSD value	**5.781 ***	

*Means with different big letters in the same column and small letters in the same row are significantly different, * (P≤0.05).

2.4. Evaluation of pain in rabbits

2.4.1. Grimace scale

In the evaluation of pain in rabbits using the Grimace Scale as in table (4), the statistical analysis revealed significant differences between the treatment groups in all observed facial features (whisker position, ear position, and eyes tightly closed) as in figure 1, specifically, in the treatment with Tramadol and Remifentanil showed comparable effects on pain, with no statistically significant difference observed between these two treatment groups across all facial features. This indicates that both drugs had a similar efficacy in pain management as measured by the Grimace Scale, in contrast, the Lidocaine treatment group showed a statistically significant reduction in the Grimace Scale scores for whisker position, ear position, and eyes tightly closed, indicating a lower pain response compared to the Tramadol and Remifentanil groups.

Further enhancements in pain reduction were observed with the addition of epinephrine or Carbopol gel to Remifentanil. Both combinations resulted in a statistically significant decrease in Grimace Scale scores for all measured features when compared to the Tramadol and Remifentanil groups, suggesting an improved analgesic effect. Within each treatment group, there was a consistent response across all facial features assessed, indicating no significant difference within each treatment group's measured parameters.

2.4.2. Behavioral analysis

In the behavioral analysis of pain assessment in rabbits as in table (5), our investigation revealed significant differences in pain responses as measured by activities and body posture, which were inferred from the mean ± SE of behavioral analysis markers. These differences were evident when comparing the effects of various analgesic treatments. Rabbits treated with Tramadol and Remifentanil exhibited higher behavioral scores, indicating a higher level of pain response. The similarity in scores suggests comparable effectiveness between these two treatments in managing pain-related behaviors.

In comparison, with tramadol and remifentanil, Lidocaine administration resulted in a significantly lower pain response, as demonstrated by reduced behavioral scores across all parameters. This finding suggests Lidocaine's superior efficacy in alleviating pain behaviors compared to Tramadol and Remifentanil. Further significant reductions in pain responses were observed with the addition of epinephrine or Carbopol gel to Remifentanil. These combinations showed the lowest behavioral scores, indicating the most substantial pain mitigation and suggesting an enhanced analgesic effect when Remifentanil is combined with these agents. There were no statistically significant variances within groups, implying that each treatment produced a consistent effect across all behavioral parameters assessed shown in Figure 1.

Table 4. Effect of tramadol, remifentanil, lidocaine, remifentanil with epinephrine and remifentanil with carbopol gel on Grimace Scale

Groups/ Drug	Mean ± SE Whisker position	Ear position	Eyes tightly
Tramadol	2.00 ±0.25 A a	2.00 ±0.25 A a	2.00 ±0.25 A a
Remifentanil	2.00 ±0.25 A a	2.00 ±0.25 A a	2.00 ±0.25 A a
Lidocaine	0.80 ±0.11 A a	0.80±0.11 A a	0.80±0.11 A a
Remifentanil with epinephrine	0.40 ±0.08 A a	0.40 ±0.08 A a	0.40 ±0.08 A a
Remifentanil with Carbopol gel	0.40 ±0.08 A a	0.40 ±0.08 A a	0.40 ±0.08 A a
LSD value		0.702 *	

*Means with different big letters in the same column and small letters in the same row are significantly different, * (P≤0.05).

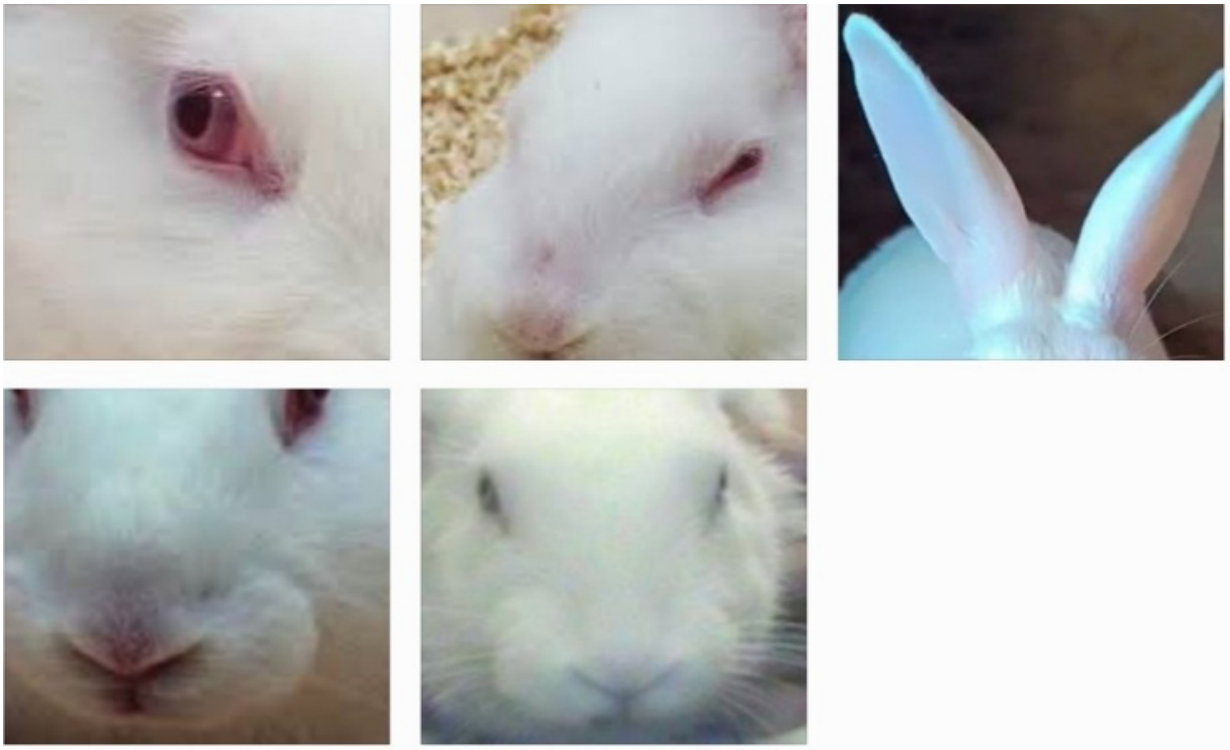

Figure 1. Facial expressions of pain in rabbits examples orbital tightening, ears position ,whisker position, nose position

Table 5. Effect of tramadol, remifentanil, lidocaine, remifentanil with epinephrine and remifentanil with carbopol gel on behavioral analysis.

Groups/ Drug	Mean ± SE		
	Activities	Vaculaization	Body posture
Tramadol	2.00 ±0.25	2.00 ±0.25	2.00 ±0.25
	A a	A a	A a
Remifentanil	2.00 ±0.25	2.00 ±0.25	2.00 ±0.25
	A a	A a	A a
Lidocaine	0.80 ±0.11	0.80±0.11	0.80±0.11
	A a	A a	A a
Remifentanil with epinephrine	0.40 ±0.08	0.40 ±0.08	0.40 ±0.08
	A a	A a	A a
Remifentanil with Carbopol gel	0.40 ±0.08	0.40 ±0.08	0.40 ±0.08
	A a	A a	A a
LSD value		0.702 *	

*Means with different big letters in the same column and small letters in the same row are significantly different, * (P≤0.05).

3. DISCUSSION

Based on the results, it can be observed that the Tramadol group experienced a significant decrease in temperature after injection, indicating a cooling effect of the drug [31]. However, the Remifentanil and Lidocaine groups showed no significant change in temperature post-injection [32]. The Remifentanil with epinephrine group had decrease in temperature but it was not statistically significant. Similarly, remifentanil with carbopol gel did not experience significant changes in temperature. The lack of significant changes in temperature in the Remifentanil could be absence of direct effects on thermoregulation. The absence of significant changes in temperature of lidocaine suggests didn't affect directly on thermoregulation. They reported a significant decrease in body temperature following Lidocaine administration [33], which contradicts the findings in the Lidocaine group in this study. Possible explanations for this discrepancy include variations in patient characteristics and the use of different routes of administration [34].

Tramadol, as mentioned earlier, is an opioid analgesic that acts as a weak agonist of the mu-opioid receptors. It also inhibits the reuptake of norepinephrine and serotonin. These actions primarily affect pain perception and modulation rather than directly influencing heart rate [35]. Remifentanil, a potent opioid analgesic, acts as a selective mu-opioid receptor agonist. It produces analgesia and sedation without significant effects on the cardiovascular system. The lack of a significant change in heart rate in the Remifentanil group aligns with its mechanism of action [36]. Lidocaine, a local anesthetic, primarily acts by blocking sodium channels and preventing nerve impulse conduction. It does not have direct effects on heart rate regulation. Reasons for No Significant Changes [37].

The slight increase in heart rate observed in the Tramadol group, although not statistically significant, could be due to indirect factors such as Misuse Disorder Potential, Genetic Variability and Metabolism, Risk Factors and Pre-existing Conditions. Tramadol's effects on pain relief and modulation may lead to a mild increase in sympathetic activity, resulting in a minor elevation in heart rate [38]. Remifentanil's selective action on mu-opioid receptors primarily affects pain perception and sedation rather than directly influencing heart rate. Therefore, the minor decrease in heart rate observed in the Remifentanil group could be attributed to other factors or individual variations [39]. Lidocaine, being a local anesthetic, does not have direct effects on heart rate regulation. Therefore, the absence of significant changes in heart rate in the Lidocaine group is expected.

Previous study investigated the effects of Tramadol on heart rate in patients with moderate to severe pain [40]. Researchers reported that non-statistically significant changes in heart rate following Tramadol administration were obtained [41], consistent with the findings in the Tramadol group in this study. In line with the lack of significant heart rate changes in the Remifentanil group, previous study examined the cardiovascular effects of Remifentanil during labor analgesia [42]. They found no significant alterations in heart rate with Remifentanil administration. On the other hand, other studies explored the effects of Lidocaine on heart rate during dental procedures, they reported a significant decrease in heart rate following Lidocaine administration, contrasting the findings in the Lidocaine group in this study [43,44]. Variations in the study population, dosage, and route of administration could account for this discrepancy.

Tramadol, as an opioid analgesic, primarily acts on the mu-opioid receptors and inhibits the reuptake of norepinephrine and serotonin. Its main effects are related to pain modulation rather than direct influence on respiratory rate. Remifentanil, a potent opioid analgesic, selectively activates the mu-opioid receptors. It produces analgesia and sedation but does not have direct effects on respiratory rate regulation (45). Lidocaine, a local anesthetic, primarily acts by blocking sodium channels and preventing nerve impulse conduction. It does not have direct effects on respiratory rate regulation. Reasons for No Significant Changes. The lack of significant changes in respiratory rate in the Tramadol group may be attributed to the drug's mechanism of action. Tramadol's effects on pain modulation are not expected to directly influence respiratory rate.

Remifentanil's selective action on mu-opioid receptors primarily affects pain perception and sedation rather than directly influencing respiratory rate. Therefore, the absence of significant changes in respiratory rate in the Remifentanil group aligns with its mechanism of action. Lidocaine, being a local anesthetic, does not have direct effects on respiratory rate regulation. Therefore, the lack of significant changes in respiratory rate in the Lidocaine group is expected.

A recently published study investigated the effects of Tramadol on respiratory parameters in patients with acute pain. They reported no significant changes in respiratory rate following Tramadol administration, consistent with the findings in the Tramadol group in this study [46]. In line with the lack of significant respiratory rate changes in the Remifentanil group, a study by Benito et al., 2019 [47] examined the respiratory effects of Remifentanil during anesthesia induction. They found no significant alterations in respiratory rate with Remifentanil administration. On the other hand, a study conducted by Reabel in 2021 explored the effects of Lidocaine on respiratory parameters during general anesthesia. They reported a significant decrease in respiratory rate following Lidocaine administration, contrasting the findings in the Lidocaine group in this study [48]. Variations in the study population, dosage, and route of administration could account for this discrepancy.

The study results indicate that Tramadol and Remifentanil had comparable effects on pain management in rabbits, as measured by the Grimace Scale. There was not statistically significant difference between these two treatment groups across all observed facial features. This suggests that both drugs' effects in reduce pain in rabbits. Lidocaine treatment. Showed significant difference decrease in Grimace Scale scores for all measured facial features compared to the Tramadol and Remifentanil group. Previous study evaluated the efficacy of Tramadol and Remifentanil in reducing pain in rabbits using the Grimace Scale. They found no statistically significant difference between Tramadol and Remifentanil in terms of Grimace Scale scores, consistent with the findings in this study [49]. In line with the enhanced effects of combining Remifentanil with epinephrine or gel, a study by Reabel in 2021 investigated the analgesic effects of Remifentanil combined with carbopol gel in rabbits. They reported a significant decrease in pain scores when Remifentanil was combined with gel, supporting the findings in this study [50].

The study findings suggest that both Tramadol and Remifentanil were associated with higher behavioral scores, indicating a higher level of pain response in rabbits. This suggests that these treatments may have a moderate effect on pain behaviors. The administration of Lidocaine resulted in significantly lower pain responses, as demonstrated by reduced behavioral scores across all parameters. This indicates that Lidocaine had a superior efficacy in alleviating pain behaviors compared to Tramadol and Remifentanil. Enhanced effects of remifentanil with epinephrine and gel. These combinations showed a significant decrease in pain responses including lowering behavior scores. These findings suggested that additional of these agents enhanced the effects and improved the pain management in rabbits within treatment groups. The lack of statistically significant variances within treatment groups implies that each treatment consistently produced effects across all assessed behavioral parameters. This indicates that the observed differences in pain responses were not specific to certain behaviors but rather represented a comprehensive response to the treatments.

In previous study, researchers evaluated the effects of Tramadol and Remifentanil on pain behaviors in rabbits using similar behavioral analysis markers. They found that both Tramadol and Remifentanil were associated with higher pain scores, consistent with the findings in this study. They also investigated the analgesic effects of Remifentanil combined with carbopol gel in rabbits. They reported a significant decrease in pain behaviors when Remifentanil was combined with gel, supporting the findings in this study [51].

4. CONCLUSION

It was concluded that tramadol and remifentanil showed a comparable anaesthetic effect that improved significantly with epinephrine or carbopol gel as local anaesthetics that surpassed lidocaine local anaesthetic effect significantly.

5. MATERIALS AND METHODS

5.1. Animals of the experiment

The experiment took place at the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad. The study involved twenty-five healthy adult male rabbits, each weighing approximately 1.5 kg. These rabbits were obtained from local markets and placed in the university's animal facility. The rabbits were kept in controlled environmental conditions, with temperatures ranged from 20-25 ^{o} C. Their habitat was an air-conditioned room with a 12-hour light cycle. They were housed in metal cages measuring 20 x 50 x 75 cm. Their diet consisted of a specially prepared pellet ration. All rabbits were given a minimum of two weeks to acclimate before the commencement of the experiment. Ethical approval was obtained prior to the study. Rabbits divided into five groups receiving different local aesthetic agents. Vital physical parameters, including temperature, respiratory rate, and heart rate, were recorded before and after administering the drugs.

5.1.1. Health Conditions

The rabbits were described as "healthy adult male". This suggests they were free from any known health issues before the start of the experiment. Any changes in their health during the experiment would depend on the specific procedures and treatments applied.

5.1.2. Deworming Process

The rabbits were likely kept at a stable temperature, either through their environment or by controlling their food intake.

5.2. Location of the study and ethical committee

Before starting this study, Ethical approval was granted through the local animal care committee and use, collage of Veterinary Medicine University of Baghdad (number P.G. 115).

5.3. Experimental design

The study involved 25 rabbits, divided into five groups as follows at animals House of collage of Veterinary Medicine University of Baghdad and received the following treatment subcutaneously:

- G1: 2% lidocaine. (4mg/kg)s/c [52].
- G2: 5% tramadol. (15mg/kg) s/c[53].
- G3: 2%remifentanil. (2mg/kg) S/c [54].
- G4: a combination of 2%remifentanil and epinephrine. (1/100.000) mg /ml S/c [55].
- G5: a Carbopol gel combined with 2% remifentanil [54].

The mucoadhesive gel was prepared according to the method described by Tugcu-Demiröz et al., (2015) [56]. Vital physical parameters, including temperature, respiratory rate, and heart rate, were meticulously recorded before and after administering local anesthetic agents. The primary objective was to assess the local impact of each drug on the experimental subjects.

5.4. Assessment of pain in rabbits

It was performed by Intracutaneous Wheel Test as follows:
Adult male rabbits who selected for the study weighted between (1.5 + 0.08) kg subjected to skin shaving with diameter 4-5 cm on the dorsal midline of rabbits. the animals were placed in a suitable restraining position. Wheels were Marked by drawing a circle around each with a marking pen. The reactions of the animals were tested to pinprick inside the wheel every minute and the time when the animal fails to respond and when it starts responding again was recorded. The animals were observed for 4 days for signs of necrosis, and ulceration, as per the method described by Geddes, 1955 [57].

5.5. Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS, 2018) software. Results presented as mean ± SE. The Least Significant Difference (LSD) test within an Analysis of Variance (ANOVA) framework was utilized to conduct comparisons between the means of the study parameters. This statistical approach was selected to determine the significance of the effects observed from the different treatments applied in this study with a significant threshold of $P \leq 0.05$ [58,59].

Acknowledgements: The authors would like to acknowledge the Department of Physiology, Biochemistry, and Pharmacology at the College of Veterinary Medicine, University of Baghdad, Iraq, for providing the facilities and resources to conduct this study.

Author contributions: Concept – R.O.; Design – N.A., R.O.; Supervision – R.O.; Resources – N.A.; Materials – N.A.; Data Collection and/or Processing – N.A.; Analysis and/or Interpretation – N.A., R.O.; Literature Search – N.A., R.O.; Writing – N.A.; Critical Reviews – R.O.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Cepeda MS, Lau J, Carr DB. Defining the therapeutic role of local anesthetic sympathetic blockade in complex regional pain syndrome: A narrative and systematic review. Clin J Pain. 2002;18(4):216-233. https://doi.org/10.1097/00002508-200207000-00002

[2] Mock CN, Donkor P, Gawande A, Jamison DT, Kruk ME, Debas HT; DCP3 Essential Surgery Author Group. Essential surgery: Key messages from Disease Control Priorities, 3rd edition. Lancet. 2015;385(9983):2209-19. https://doi.org/10.1016/s0140-6736(15)60091-5

[3] Taylor A, McLeod G. Basic pharmacology of local anaesthetics. BJA Educ. 2020;20(2):34-41. https://doi.org/10.1016%2Fj.bjae.2019.10.002

[4] Kitson A, Harvey G, McCormack B. Enabling the implementation of evidence based practice: A conceptual framework. Qual Health Care. 1998;7(3):149-158. https://doi.org/10.1136/qshc.7.3.149

[5] Bourne E, Wright C, Royse C. A review of local anesthetic cardiotoxicity and treatment with lipid emulsion. Local Reg Anesth. 2010;3:11-19. https://doi.org/10.2147%2Flra.s8814

[6] Shipton EA. New formulations of local anaesthetics-part I. Anesthesiol Res Pract. 2012;2012:546409. https://doi.org/10.1155%2F2012%2F546409

[7] Weiniger CF, Golovanevski M, Sokolsky-Papkov M, Domb AJ. Review of prolonged local anesthetic action. Expert Opin Drug Deliv. 2010;7(6):737-752. https://doi.org/10.1517/17425241003767383

[8] Wang J, Zhao G, Song G, Liu J. The efficacy and safety of local anesthetic techniques for postoperative analgesia after cesarean section: A Bayesian network meta-analysis of randomized controlled trials. J Pain Res. 2021;14:1559-1572. https://doi.org/10.2147%2FJPR.S313972

[9] Fernandez E, Milburn TW. Sensory and affective predictors of overall pain and emotions associated with affective pain. Clin J Pain. 1994;10(1):3-9. https://doi.org/10.1097/00002508-199403000-00002

[10] Craig KD, MacKenzie NE. What is pain: Are cognitive and social features core components? Paediatr Neonatal Pain. 2021;3(3):106-118. https://doi.org/10.1002/pne2.12046

[11] Yanagidate F, Strichartz GR. Local anesthetics. Handb Exp Pharmacol. 2007;(177):95-127. https://doi.org/10.1007/978-3-540-33823-9_4

[12] Dinakar K R, Sanji N, Ravishankar RB, Vidya HK, Shashikala G H. Comparative study of variations in blood pressure and heart rate among normotensive patients and hypertensive patients receiving angiotensin receptor blockers during surgery under spinal anesthesia. Natl J Physiol Pharm Pharmacol. 2018; 8(12): 1581-1586. http://dx.doi.org/10.5455/njppp.2018.8.0827106092018

[13] Dwivedi MB, Nagrale M, Dwivedi S, Singh H. What happens to the hemodynamic responses for laryngeal mask airway insertion when we supplement propofol with butorphanol or fentanyl for induction of anesthesia: A comparative assessment and critical review. Int J Crit Illn Inj Sci. 2016 6(1):40-44. https://doi.org/10.4103%2F2229-5151.177369

[14] Alappat AM, Lagoo JY, Shivappagoudar V. Evaluation of haemodynamic stability following induction of general anaesthesia with propofol and etomidate in normotensive and hypertensive patients: A comparative study. Glob J Res Anal. 2020;9(10): 117-122. http://dx.doi.org/10.36106/gjra/7510742

[15] Bhatia A, Buvanendran A. Anesthesia and postoperative pain control-multimodal anesthesia protocol. J Spine Surg. 2019;5(Suppl 2):S160-S165. https://doi.org/10.21037/jss.2019.09.33.

[16] Hosseinzadeh H, Eidy M, Golzari SE, Vasebi M. Hemodynamic stability during ınduction of anesthesia in elderly patients: Propofol + ketamine versus propofol + etomidate. J Cardiovasc Thorac Res. 2013;5(2):51-54. https://doi.org/10.5681%2Fjcvtr.2013.011

[17] Epstein-Barash H, Shichor I, Kwon AH, Hall S, Lawlor MW, Langer R, Kohane DS. Prolonged duration local anesthesia with minimal toxicity. Proc Natl Acad Sci U S A. 2009;106(17):7125-7130. https://doi.org/10.1073/pnas.0900598106

[18] Kaufman E, Epstein JB, Gorsky M, Jackson DL, Kadari A. Preemptive analgesia and local anesthesia as a supplement to general anesthesia: a review. Anesth Prog. 2005;52(1):29-38. https://doi.org/10.2344%2F0003-3006(2005)52%5B29%3APAALAA%5D2.0.CO%3B2

[19] Bagshaw KR, Hanenbaum CL, Carbone EJ, Lo KW, Laurencin CT, Walker J, Nair LS. Pain management via local anesthetics and responsive hydrogels. Ther Deliv. 2015;6(2):165-176. https://doi.org/10.4155%2Ftde.14.95

[20] Cherobin ACFP, Tavares GT. Safety of local anesthetics. An Bras Dermatol. 2020 ;95(1):82-90. https://doi.org/10.1016%2Fj.abd.2019.09.025
[21] Ring ME. The history of local anesthesia. J Calif Dent Assoc. 2007;35(4):275-82.
[22] Barakat A. Revisiting tramadol: A multi-modal agent for pain management. CNS Drugs. 2019;33(5):481-501. https://doi.org/10.1007/s40263-019-00623-5
[23] Bidwell LA, Bramlage LR, Rood WA. Equine perioperative fatalities associated with general anaesthesia at a private practice--a retrospective case series. Vet Anaesth Analg. 2007;34(1):23-30. https://doi.org/10.1111/j.1467-2995.2005.00283.x
[24] Santonocito C, Noto A, Crimi C, Sanfilippo F. Remifentanil-induced postoperative hyperalgesia: current perspectives on mechanisms and therapeutic strategies. Local Reg Anesth. 2018;11:15-23. https://doi.org/10.2147/lra.s143618
[25] Saxena S, Gonsette K, Terram W, Huybrechts I, Nahrwold DA, Cappello M, Barvais L, Engelman E. Gradual withdrawal of remifentanil delays initial post-operative analgesic demand after thyroid surgery; double-blinded, randomized controlled trial. BMC Anesthesiol. 2019;19(1):60. https://doi.org/10.1186/s12871-019-0731-9
[26] Vazzana M, Andreani T, Fangueiro J, Faggio C, Silva C, Santini A, Garcia ML, Silva AM, Souto EB. Tramadol hydrochloride: pharmacokinetics, pharmacodynamics, adverse side effects, co-administration of drugs and new drug delivery systems. Biomed Pharmacother. 2015;70:234-238. https://doi.org/10.1016/j.biopha.2015.01.022
[27] Scott LJ, Perry CM. Tramadol: a review of its use in perioperative pain. Drugs. 2000;60(1):139-176. https://doi.org/10.2165/00003495-200060010-00008
[28] Shah J, Nair AB, Shah H, Jacob S, Shehata TM, Morsy MA. Enhancement in antinociceptive and anti-inflammatory effects of tramadol by transdermal proniosome gel. Asian J Pharm Sci. 2020;15(6):786-796. https://doi.org/10.1016/j.ajps.2019.05.001
[29] Akbar H, Khan MA, Bokhari SG, Khan HM, Anjum AA. Comparative efficacy of medetomidine HCl and lignocaine HCl as epidural anesthetic in buffalo calves. Pak Vet J. 2014;34(3):377-380
[30] Kang Z, Zhu G, Su C, Zeng K, Li S, Wu X. Differential effects of remifentanil and sufentanil anesthesia on post-operative pain and cognitive functions. Int Immunopharmacol. 2022;108:108888. https://doi.org/10.1016/j.intimp.2022.108888
[31] Chinyanga HM. Temperature regulation and anesthesia. Pharmacol Ther. 1984;26(2):147-161. https://doi.org/10.1016/0163-7258(84)90014-7
[32] Jeong CW, Lee SH, Ju J, Jeong SW, Lee HG. The effect of priming injection of different doses of remifentanil on injection pain of microemulsion propofol premixed with lidocaine. Korean J Anesthesiol. 2011;60(2):78-82. https://doi.org/10.4097%2Fkjae.2011.60.2.78
[33] Jæger P, Koscielniak-Nielsen ZJ, Hilsted KL, Grevstad U, Siersma V, Fabritius ML, Dahl JB. Effect of total dose of lidocaine on duration of adductor canal block, assessed by different test methods: A report of two blinded, randomized, crossover studies in healthy volunteers. Anesth Analg. 2016;123(4):1026-1032. https://doi.org/10.1213/ane.0000000000001517
[34] Di Bona D, Plaia A, Scafidi V, Leto-Barone MS, Di Lorenzo G. Efficacy of sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: A systematic review and meta-analysis. J Allergy Clin Immunol. 2010;126(3):558-566. https://doi.org/10.1016/j.jaci.2010.06.013
[35] Ide S, Minami M, Ishihara K, Uhl GR, Sora I, Ikeda K. Mu opioid receptor-dependent and independent components in effects of tramadol. Neuropharmacology. 2006;51(3):651-658. https://doi.org/10.1016/j.neuropharm.2006.05.008
[36] Nowoczyn M, Marie N, Coulbault L, Hervault M, Davis A, Hanouz JL, Allouche S. Remifentanil produces cross-desensitization and tolerance with morphine on the mu-opioid receptor. Neuropharmacology. 2013;73:368-379. https://doi.org/10.1016/j.neuropharm.2013.06.010
[37] Yu S, Wang B, Zhang J, Fang K. The development of local anesthetics and their applications beyond anesthesia. Int J Clin Exp Med. 2019;12(12):13203-13220.
[38] Grond S, Meuser T, Zech D, Hennig U, Lehmann KA. Analgesic efficacy and safety of tramadol enantiomers in comparison with the racemate: A randomised, double-blind study with gynaecological patients using intravenous patient-controlled analgesia. Pain. 1995;62(3):313-320. https://doi.org/10.1016/0304-3959(94)00274-i
[39] Lee SK, Jeong MA, Sung JM, Yeon HJ, Chang JH, Lim H. Effect of remifentanil infusion on the hemodynamic response during induction of anesthesia in hypertensive and normotensive patients: A prospective observational study. J Int Med Res. 2019;47(12):6254-6267. https://doi.org/10.1177%2F0300060519883568
[40] Thomas RD, Behbehani MM, Coyle DE, Denson DD. Cardiovascular toxicity of local anesthetics: An alternative hypothesis. Anesth Analg. 1986;65(5):444-450.
[41] Wu TY, Wu WT, Lee RP, Chen IH, Yu TC, Wang JH, Yeh KT. tramadol may ıncrease risk of hip fracture in older adults with post-traumatic osteoarthritis. J Pers Med. 2023;13(4):580. https://doi.org/10.3390/jpm13040580
[42] Konefał H, Jaskot B, Czeszyńska MB, Pastuszka J. Remifentanil patient-controlled analgesia for labor - monitoring of newborn heart rate, blood pressure and oxygen saturation during the first 24 hours after delivery. Arch Med Sci. 2013;9(4):697-702. https://doi.org/10.5114%2Faoms.2012.31306
[43] Marques-Ferreira M, Carrilho E, Paulo S, Carrilho T, Pedro Figueiredo J, Macedo R. Anaesthesia in dental medicine with local ınfiltrative anaesthetic technique versus diploe anaesthesia delivery systems: Efficacy and behaviour, an experimental study. Acta Med Port. 2017;30(12):848-853. https://doi.org/10.20344/amp.8710
[44] Lehmann HS, Musk GC, Laurence M, Hyndman TH, Tuke J, Collins T, Gleerup KB, Johnson CB. Mitigation of electroencephalographic and cardiovascular responses to castration in Bos indicus bulls following the

administration of either lidocaine or meloxicam. Vet Anaesth Analg. 2017;44(6):1341-1352. https://doi.org/10.1016/j.vaa.2017.04.009

[45] Sabourdin N, Barrois J, Louvet N, Rigouzzo A, Guye ML, Dadure C, Constant I. Pupillometry-guided ıntraoperative remifentanil administration versus standard practice ınfluences opioid use: A randomized study. Anesthesiology. 2017;127(2):284-292. https://doi.org/10.1097/aln.0000000000001705

[46] Mansour HS, Ali NS, Abdel Rahman MA. The effect of dexamethasone as an adjuvant in quadratus lumborum block to improves analgesia after laparoscopic cholecystectomy: Controlled randomized study. Egypt J Anaesth. 2024;40(1): 135-142. http://dx.doi.org/10.1080/11101849.2024.2322902

[47] Benato L, Rooney NJ, Murrell JC. Pain and analgesia in pet rabbits within the veterinary environment: A review. Vet Anaesth Analg. 2019;46(2):151-162. https://doi.org/10.1016/j.vaa.2018.10.007

[48] Reabel SN, Queiroz-Williams P, Cremer J, Langohr IM, da Cunha AF, Hampton CE, Carossino M, Liu CC, Nevarez JG. Comparison of blind and endoscopic-guided orotracheal intubation on laryngeal and tracheal damage in domestic rabbits (*Oryctolagus cuniculus*). Vet Anaesth Analg. 2022;49(4):398-406. https://doi.org/10.1016/j.vaa.2022.04.003

[49] Ozawa S, Cenani A, Sanchez-Migallon Guzman Lv D. Treatment of pain in rabbits. Vet Clin North Am Exot Anim Pract. 2023;26(1):201-227. https://doi.org/10.1016/j.cvex.2022.09.001

[50] Reabel SN, Queiroz-Williams P, Cremer J, Hampton CE, Liu CC, da Cunha A, Nevarez JG. Assessment of intramuscular administration of three doses of alfaxalone combined with hydromorphone and dexmedetomidine for endoscopic-guided orotracheal intubation in domestic rabbits (*Oryctolagus cuniculus*). J Am Vet Med Assoc. 2021;259(10):1148-1153. https://doi.org/10.2460/javma.20.07.0402

[51] Guedes PEB, Pinto TM, Corrêa JMX, Niella RV, Dos Anjos CM, de Oliveira JNS, Marques CSDC, de Souza SS, da Silva EB, de Lavor MSL. Efficacy of preemptive analgesia with amantadine for controlling postoperative pain in cats undergoing ovariohysterectomy. Animals (Basel). 2024;14(4):643. https://doi.org/10.3390%2Fani14040643

[52] Abdullah S, Tokiran MF, Ahmad AA, Soh EZF, Makpol S, Sapuan J. Safety of lidocaine during wide-awake local anesthesia no tourniquet for distal radius plating. J Hand Surg Glob Online. 2023;5(2):196-200. https://doi.org/10.1016/j.jhsg.2022.12.003

[53] Anderson TJ, Grégoire J, Pearson GJ, Barry AR, Couture P, Dawes M, Francis GA, Genest J Jr, Grover S, Gupta M, Hegele RA, Lau DC, Leiter LA, Lonn E, Mancini GB, McPherson R, Ngui D, Poirier P, Sievenpiper JL, Stone JA, Thanassoulis G, Ward R. 2016 Canadian Cardiovascular Society Guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2016;32(11):1263-1282. https://doi.org/10.1016/j.cjca.2016.07.510

[54] Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature. 2001;414(6860):212-216. https://doi.org/10.1038/35102591

[55] Reed KL, Malamed SF, Fonner AM. Local anesthesia part 2: technical considerations. Anesth Prog. 2012 ;59(3):127-136; quiz 137. https://doi.org/10.2344%2F0003-3006-59.3.127

[56] Tuğcu-Demiröz F, Acartürk F, Özkul A. Preparation and characterization of bioadhesive controlled-release gels of cidofovir for vaginal delivery. J Biomater Sci Polym Ed. 2015;26(17):1237-1255. https://doi.org/10.1080/09205063.2015.1082808

[57] Geddes IC. Studies with local anaesthetics/I Review of the Literature concerning the methods of comparing Local Anaesthetic Properties. Brit J Anaesth. 1955;27(12): 609 – 615.

[58] Jabur MS, Manna MJ, Mohammed HR, Baqir LS, Abdulamir HA. Ocular hypotensive effect for the topical amlodipine 0.5% eye drop. Lat Am J Pharm. 2023;42(special issue): 311-314.

[59] Abdulhusseın HA, Alwasıtı EAR, Khıro NK, Nile AK. The potential impact of vascular endothelial growth factor rs699947 polymorphisms on breast tumors susceptibility in a sample of Iraqi females. Acta Pharm Sci. 2024;62:(2): 268-277. https://doi.org/10.23893/1307-2080.APS6217