

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

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ABSTRACT: Tramadol and remifentanyl, 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 ± 0.08 g) who were divided into five groups received the following treatment subcutaneously for 5 days; G1: 5% Tramadol 15 mg/kg BW; G2: 2% Remifentanyl 2 mg/kg BW; G3: 2% Lidocaine 4 mg/kg BW; G4: 2% Remifentanyl with epinephrine 0.001 mg/kg BW; and G5: 2% Remifentanyl 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 remifentanyl. 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 Remifentanyl groups and further significant improvement in pain were observed with the addition of epinephrine or Carbopol gel to Remifentanyl. Both combinations resulted in a statistically significant decrease in pain parameters for all measured features comparing with Tramadol and Remifentanyl groups and these results leads to conclude that tramadol and remifentanyl 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; remifentanyl; 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

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is refractory to alternative approaches [13]. Local anesthetics result in a slight increase in heart rate (HR) due 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 remifentanyl 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 remifentanyl 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 remifentanyl 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 $39.15 \pm 0.06^{\circ}\text{C}$ to $38.27 \pm 0.24^{\circ}\text{C}$), indicating that the drug had a cooling effect. On the other hand, the Remifentanyl group showed a non-significant ($p > 0.05$) change in temperature post-injection ($39.12 \pm 0.06^{\circ}\text{C}$ to $39.10 \pm 0.04^{\circ}\text{C}$). The Lidocaine group also showed a non-significant change and rabbits received Remifentanyl with epinephrine or carbopol gel also showed a non-significant change in the temperatures.

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 Remifentanyl Lidocaine and remifentanyl with carbopol gel group. The Remifentanyl with epinephrine group also had a lower temperature compared to the Remifentanyl group, but this was not statistically significant. There was no significant difference in the temperature change after injection between the Remifentanyl, Lidocaine, and Remifentanyl with carbopol gel groups. The purpose of this addition for prolonged duration of action for remifentanyl.

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

| Groups/ Drug | Mean \pm SE of Temperature | |
|--------------------------------|------------------------------|-------------------------|
| | Before injection (at 0 time) | After injection |
| Tramadol | 39.15 \pm 0.06 A a | 38.27 \pm 0.24 B b |
| Remifentanyl | 39.12 \pm 0.06 A a | 39.10 \pm 0.04 A a |
| Lidocaine | 39.15 \pm 0.06 A a | 39.12 \pm 0.07 A a |
| Remifentanyl with epinephrine | 39.15 \pm 0.06 A a | 38.62 \pm 0.24 A a |
| Remifentanyl with Carbopol gel | 39.07 \pm 0.04 A a | 39.10 \pm 0.05 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 \leq 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 Remifentanyl group experienced a minor decrease in heart rate post-injection, which was also not statistically significant (from 142.33 \pm 0.88 bpm to 141.33 \pm 0.67 bpm). No significant change in heart rate was observed in the Lidocaine group (143.33 \pm 1.76 bpm to 142.33 \pm 1.76 bpm). The Remifentanyl with epinephrine group's heart rate remained essentially unchanged after injection (141.00 \pm 0.57 bpm to 141.33 \pm 0.33 bpm). Similarly, the Remifentanyl with carbopol gel group showed no significant change in heart rate (141.33 \pm 0.67 bpm to 141.33 \pm 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 injection on heart rate measures in rabbits

| Groups/ Drug | Mean \pm SE of Heart rate | |
|--------------------------------|-----------------------------|--------------------------|
| | Before drug | After injection drug |
| Tramadol | 143.00 \pm 1.52 A a | 144.00 \pm 0.57 A a |
| Remifentanyl | 142.33 \pm 0.88 A a | 141.33 \pm 0.67 A a |
| Lidocaine | 143.33 \pm 1.76 A a | 142.33 \pm 1.76 A a |
| Remifentanyl with epinephrine | 141.00 \pm 0.57 A a | 141.33 \pm 0.33 A a |
| Remifentanyl with Carbopol gel | 141.33 \pm 0.67 A a | 141.33 \pm 0.88 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 \pm 3.17 to 45.00 \pm 3.00). The Remifentanyl group also showed no significant change in respiratory rate (from 38.67 \pm 0.33 to 38.33 \pm 0.33). No significant change in respiratory rate was observed in the Lidocaine group (from 41.33 \pm 3.38 to 41.67 \pm 2.73). The Remifentanyl with epinephrine group's respiratory rate decreased slightly, but not significantly (from 42.00 \pm 3.51 to 38.67 \pm 0.33). Similarly, the Remifentanyl with carbopol gel group showed no significant change in respiratory rate (from 38.00 \pm 1.00 to 38.33 \pm 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 \pm SE of Respiratory rate | |
|--------------------------------|-----------------------------------|--------------------------|
| | Before drug | After injection drug |
| Tramadol | 45.33 \pm 3.17 A a | 45.00 \pm 3.00 A a |
| Remifentanyl | 38.67 \pm 0.33 B a | 38.33 \pm 0.33 B a |
| Lidocaine | 41.33 \pm 3.38 AB a | 41.67 \pm 2.73 AB a |
| Remifentanyl with epinephrine | 42.00 \pm 3.51 AB a | 38.67 \pm 0.33 B a |
| Remifentanyl with Carbopol gel | 38.00 \pm 1.00 B a | 38.33 \pm 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, * ($P \leq 0.05$).

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 Remifentanyl 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 Remifentanyl groups.

Further enhancements in pain reduction were observed with the addition of epinephrine or Carbopol gel to Remifentanyl. Both combinations resulted in a statistically significant decrease in Grimace Scale scores for all measured features when compared to the Tramadol and Remifentanyl 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 \pm SE of behavioral analysis markers. These differences were evident when comparing the effects of various analgesic treatments. Rabbits treated with Tramadol and Remifentanyl 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 remifentanyl, 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 Remifentanyl. Further significant reductions in pain responses were observed with the addition of epinephrine or Carbopol gel to Remifentanyl. These combinations showed the lowest behavioral scores, indicating the most substantial pain mitigation and suggesting an enhanced analgesic effect when Remifentanyl 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, remifentanyl, lidocaine, remifentanyl with epinephrine and remifentanyl with carbopol gel on Grimace Scale

| Groups/ Drug | Whisker position | Mean ± SE | |
|--------------------------------|-------------------|-------------------|-------------------|
| | | Ear position | Eyes tightly |
| Tramadol | 2.00 ±0.25 A a | 2.00 ±0.25 A a | 2.00 ±0.25 A a |
| Remifentanyl | 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 |
| Remifentanyl with epinephrine | 0.40 ±0.08 A a | 0.40 ±0.08 A a | 0.40 ±0.08 A a |
| Remifentanyl 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).

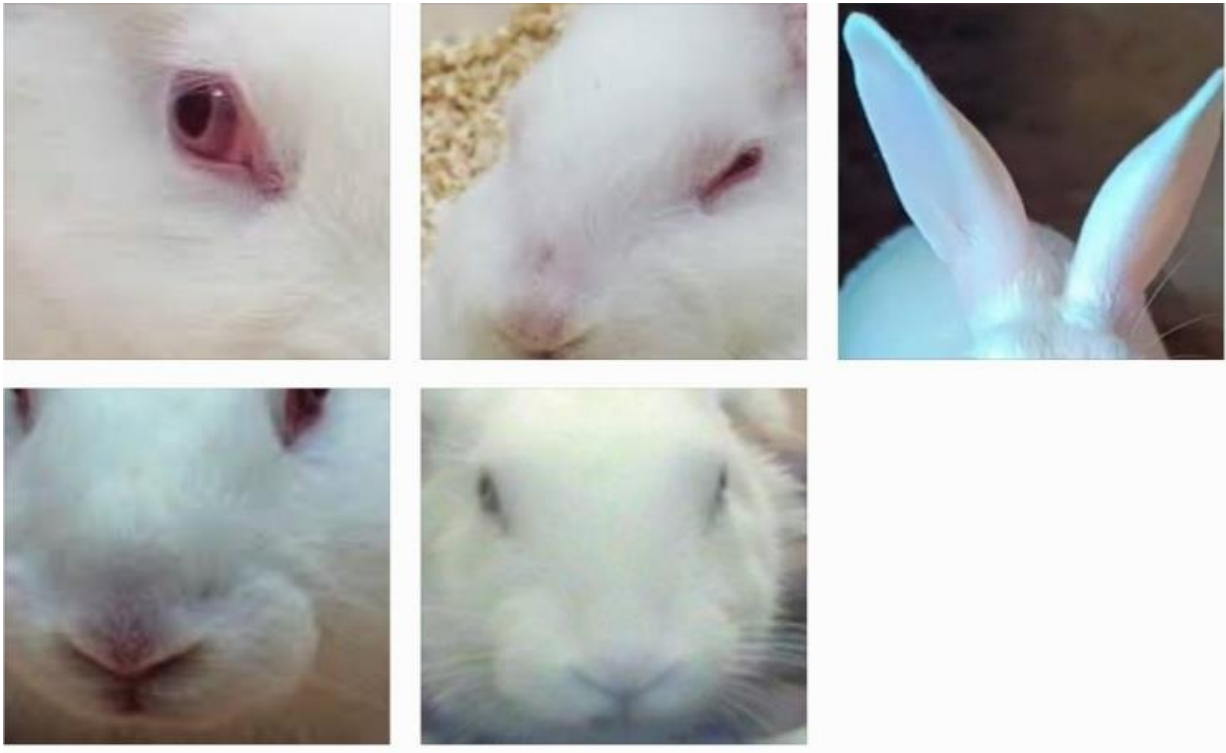


Figure 1. Facial expressions of pain in rabbits examples orbital tightening, ears position ,whisker position, nose position

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

| Groups/ Drug | Activities | Mean \pm SE | |
|--------------------------------|------------------------|------------------------|------------------------|
| | | Vaculaization | Body posture |
| Tramadol | 2.00 \pm 0.25 A a | 2.00 \pm 0.25 A a | 2.00 \pm 0.25 A a |
| Remifentanyl | 2.00 \pm 0.25 A a | 2.00 \pm 0.25 A a | 2.00 \pm 0.25 A a |
| Lidocaine | 0.80 \pm 0.11 A a | 0.80 \pm 0.11 A a | 0.80 \pm 0.11 A a |
| Remifentanyl with epinephrine | 0.40 \pm 0.08 A a | 0.40 \pm 0.08 A a | 0.40 \pm 0.08 A a |
| Remifentanyl with Carbopol gel | 0.40 \pm 0.08 A a | 0.40 \pm 0.08 A a | 0.40 \pm 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, * ($P \leq 0.05$).

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 Remifentanyl and Lidocaine groups showed no significant change in temperature post-injection [32]. The Remifentanyl with epinephrine group had decrease in temperature but it was not statistically significant. Similarly, remifentanyl with carbopol gel did not experience significant changes in temperature. The lack of significant changes in temperature in the Remifentanyl 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]. Remifentanyl, 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 Remifentanyl 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]. Remifentanyl'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 Remifentanyl 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 Remifentanyl group, previous study examined the cardiovascular effects of Remifentanyl during labor analgesia [42]. They found no significant alterations in heart rate with Remifentanyl 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. Remifentanyl, 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.

Remifentanyl'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 Remifentanyl 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 Remifentanyl group, a study by Benito et al., 2019 [47] examined the respiratory effects of Remifentanyl during anesthesia induction. They found no significant alterations in respiratory rate with Remifentanyl 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 Remifentanyl 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 Remifentanyl group. Previous study evaluated the efficacy of Tramadol and Remifentanyl in reducing pain in rabbits using the Grimace Scale. They found no statistically significant difference between Tramadol and Remifentanyl in terms of Grimace Scale scores, consistent with the findings in this study [49]. In line with the enhanced effects of combining Remifentanyl with epinephrine or gel, a study by Reabel in 2021 investigated the analgesic effects of Remifentanyl combined with carbopol gel in rabbits. They reported a significant decrease in pain scores when Remifentanyl was combined with gel, supporting the findings in this study [50].

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

4. CONCLUSION

It was concluded that tramadol and remifentanyl 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 ° 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%remifentanyl. (2mg/kg) S/c [54].
- G4: a combination of 2%remifentanyl and epinephrine. (1/100.000) mg /ml S/c [55].
- G5: a Carbopol gel combined with 2% remifentanyl [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 \pm 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].

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