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Duration of cuteneous analgesia induced by bupivacaine and ketamine in female Wistar rats; role of epinephrine

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ABSTRACT

Background: This study was designed to investigate the cumulative effect of bupivacaine and ketamine on rats' cutaneous trunci muscle reflex (CTMR) and the effect of epinephrine on the duration of analgesia.
Methods: Fifteen rats were randomly selected into three groups of five rats each. Each rat served as its own control with the right and left thoracolumbar regions being the experimental and control sites respectively. Rats in the first group (group A) were administered Bupivacaine alone, Group B rats were given Ketamine alone while Group C rats were infiltrated with a combination of Bupivacaine and Ketamine. The experiment was repeated seven days after with the addition of epinephrine to all the 3 categories of infiltrate. 0.6 ml total volume of drugs in each group was injected subcutaneously following which CTMR was stimulated via forceps bite on the injection and control sites alternately. The duration of induced analgesia (period of negative responses) in each group was recorded.
Results: The result showed a statistically significant difference ($P < 0.05$) in the duration of analgesia among the three groups. Bupivacaine and ketamine injections individually produced analgesia of 160 min and 120 min, respectively while a combination of both drugs acted for less duration. The duration of analgesia was further prolonged in all three groups following the addition of epinephrine.
Conclusion: Bupivacaine and ketamine have a combined analgesic effect that is permissive on one another rather than additive and these effects are prolonged by concurrent administration with epinephrine due to its local vasoconstrictive effect.

Introduction

Analgesics are drugs that relieve pain selectively without blocking the conduction of nerve impulses, but markedly altering sensory

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perception or affecting consciousnesses.' Analgesics can be classified into two major groups, namely; non-opioid analgesics which include nonsteroidal anti-inflammatory drugs (NSAID), and Opioid analgesics [1]. Anti-inflammatory analgesic drugs work by reducing local inflammatory responses, while opioids act on the central nervous system. Opioids are also called narcotic drugs because they induce sleep, and can be used for either short-term or long-term relief of severe pain. Analgesics serve many clinical purposes, including the prevention or blockage of pain sensation during surgery and in relieving pain and inflammation after surgery.

Ketamine hydrochloride is an injectable medication used mainly for surgical anesthesia. Ketamine is commonly used along with other anesthetic agents during surgery but can be used alone for pain control during certain procedures that do not require muscle relaxation. It impacts certain nervous system functions by inhibiting normal N-Methyl-D-aspartate [NMDA] receptor activity [2]. The NMDA receptors which are located on the surface of nerve cells bind to neurotransmitters to modulate some actions of the nervous system. Ketamine is an NMDA receptor antagonist that blocks the receptors thereby rapidly diminishing pain sensation, induces sleep, and inhibits memory. This drug can cause a sense of dissociation from reality and may lead to fleeting hallucinations. Ketamine acts rapidly after administration with effects seen within seconds, and these effects wear off within 15–20 min. These actions may be modified in patients with certain morbidities including liver or kidney impairment.

Bupivacaine is a local anaesthetic agent. It can be used in epidural injection into the epidural space for epidural anaesthesia and analgesia during labor, surgery, or certain medical procedures. Bupivacaine blocks nerve impulses that send pain signals to the brain. Bupivacaine has a slow onset of action (about 5–10 min after injection), but its effect lasts longer for about 4–8 h.

Epinephrine is a hormone and neurotransmitter with multiple therapeutic importance. It is used in the management of allergic reactions, cardiac arrhythmias and also in the control of mucosa congestion, glaucoma, and asthma. Epinephrine is of key importance in the management of anaphylaxis and hypotension in patients suffering from septic shock [3]. Epinephrine acts on both alpha- and beta-adrenergic receptors. Through its action on alpha-adrenergic receptors, epinephrine minimizes the vasodilation and increased vascular permeability that occurs during anaphylaxis which can cause the loss of intravascular fluid volume as well as hypotension. Following intravenous, subcutaneously, or intramuscular injection, epinephrine has a rapid onset of action, a short duration of action, and disappears rapidly from the bloodstream.

The administration of ketamine or bupivacaine in larger volumes has been reported to frequently result in side effects including; loss of fine judgment, dizziness, hallucination, slurred speech, diminished reflexes, nystagmus, and depressed mental state. The risk of these side effects tend to increase as the concentration administered is increased, which is one of the key basis for combination therapy since a combination of two drugs will invariably mean a lower volume of each of the drugs. This present study is designed to investigate the duration of cutaneous analgesia produced by ketamine and bupivacaine individually and when combined and also assess the role played by the addition of epinephrine to each drug preparation.

Material and methods

Animals

Fifteen female Wistar rats were used for this study, each weighing between 150 and 200g. The rats were procured from the Animal House of the Physiology Department at Osun State University, Osogbo, Osun State, Nigeria. The animals were randomly divided into three groups each containing five rats and were kept in wooden cages with iron mesh, at standard condition. They were fed with rat pellets and water ad libitum. The rats were left for two weeks to acclimatize to the study environment. All procedures involving the use of animals in this study complied with the guidelines for research involving animals as reaffirmed by the ethical principles underlying the Declaration of Helsinki, and the Guidelines for the care and use of animals [4].

Study design and administration of drugs

The drugs tested (Bupivacaine, Ketamine, Epinephrine) were injected using 21G x 1.5″ gauge needle, subcutaneously in unanaesthetised rats under the dorsal surface of the thoracolumbar region, from which hair was removed (via razor blade shaving) 24 h before the experiments. The circular elevation of the skin caused by the injection (a wheal) was immediately marked with ink.

Each drug (with or without epinephrine) or combination of drugs tested was injected into a naïve area of the rat's shaved back. The back was divided into two halves to clearly demarcate injection and control sites, and each animal in each group was injected twice with the drug being tested for the group (Once without epinephrine and once with epinephrine) separated by an interval of 7 days.

All drug combinations were given as a single injection in a volume of 0.6 ml same as single drug injections.

Each rat in the respective groups ($n = 5$ for each group) was injected with a single injection of 0.6 ml of the following drugs (weight/volume [%]);

1. GROUP A

EXP. 1; 0.6 ml of 0.05 % racemic bupivacaine
EXP. 2; 0.6 ml of 0.05 % racemic bupivacaine plus epinephrine (1:1000), 0.05mg/Kg

2. GROUP B

EXP. 1; 0.6 ml of (0.5mg/kg BW Ketamine) plus sterile water
EXP. 2; 0.6 ml of (0.5mg/kg BW Ketamine) plus epinephrine (1:1000), 0.05mg/Kg plus sterile water

3. GROUP C

EXP. 1; 0.6 ml of 0.05 % racemic bupivacaine plus 0.5mg/kg BW Ketamine
EXP. 2; 0.6 ml of 0.05 % racemic bupivacaine plus 0.5mg/kg BW Ketamine plus epinephrine (1:1000), 0.05mg/Kg

Preparation and dosage of bupivacaine

Bupivacaine purchased from Alchem Inc, Minnesota, USA was used for this study. 1 ml of 0.5 %. It was diluted with 9 ml of sterile water to get 0.05 % of bupivacaine. 0.6 ml of this was infiltrated [5].

Preparation and dosage of ketamine

The dosage of ketamine used was 0.5mg/kg Body weight [6]. 1 ml of 50mg/ml Ketamine solution was dissolved in 200 ml of sterile water to get a stock solution of 0.25mg/ml. Each rat was given (0.002 ml x BW) of the stock solution.

Preparation and dosage of epinephrine

The dosage of epinephrine used was 0.05mg/Kg [7]. 0.5 ml of 1mg/ml (1:1000) epinephrine was dissolved in 10 ml of sterile water to produce a stock solution with a concentration of 0.05mg/ml (1:200,000). Each rat was injected with 0.001 ml x BW) of this stock solution.

Assessment of the duration of analgesia

The duration of analgesia induced by bupivacaine, bupivacaine + epinephrine, Ketamine, Ketamine + epinephrine, or the combination drugs plus/minus epinephrine was assessed through repeated attempts at stimulating cutaneous trunci muscle reflex (CTMR) via forceps bites on both the injection site and the control site in alternate fashion. CTMR, produced by twitches of the lateral thoracospinal muscles in response to local dorsal cutaneous stimulation, was studied as a reaction to painful forceps bites.

This procedure essentially evaluated the extent and duration of inhibition of CTMR produced by subcutaneous injection of 0.6 ml of each drug or drug combination**.** After observing the animal's normal reaction to five forcep bites on the contralateral control side, five forcep bites were applied inside the wheal, and the number to which the rat reacted was recorded. Five forcep bites was done per test to obtain reproducible results among all five rats within study groups and the test was repeated after every 20 min until fully normal response was recorded from injection sites.

The effectiveness and duration of the induced local analgesia were evaluated quantitatively as the number of times the forcep bite elicited a positive CTMR response as follows:

- Complete absence of five responses was defined as a complete nociceptive block (i.e. 0 % pain or maximum possible effect [MPE])
- The presence of one response of five was scored as 20 % pain.
- The presence of two responses of five was scored as 40 % pain.
- The presence of three responses of five as scored as 60 % pain.
- The presence of four responses of five was scored as 80 % pain, and
- Responses identical to the control responses was recorded as 100 % pain.

The observer was always blinded to the type of drug injected into the injection site.

Statistical analysis

The results were subjected to statistical analysis using MS Excel 2013. Data was expressed as Mean ± SEM. One-way ANOVA was used to analyze differences between groups and $P < 0.05$ was considered statistically significant.

Table 1
Duration and corresponding percentage CTMR responses on control and injection sites following bupivacaine injection in female Wistar rats.

	Response (%)								
Duration (Mins)	0	20	40	60	80	100	120	140	160
Control	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0
BUP	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	20±0*	52±5*	100±0

Values are expressed as the Mean ± Standard Error of Mean, $n = 5$,.
* $P < 0.05$ vs control, BUP = Bupivacaine.

Results

Effect of bupivacaine on cutaneous trunci muscle reflex (CTMR)

Table 1 shows the duration and percentage response from both injection and control sites after the administration of bupivacaine. Zero percent (0 %) response was recorded from injection sites during the first 100 min which was significantly different from the 100 % response recorded from the control ($p < 0.05$). Minimal response was recorded at 120 and 140 min but these were still significantly different when compared to the responses from control sites. 100 % response was recorded from injection sites at 160 min which was the same as that recorded from control sites.

Effect of ketamine on cutaneous trunci muscle reflex (CTMR)

Table 2 shows the duration and percentage response from both injection and control sites after the administration of ketamine. Zero percent (0 %) response was recorded from injection sites during the first 40 min which was significantly different from the 100 % response recorded from the control ($p < 0.05$). Minimal response was recorded at 60 and 100 min but these were still significantly different when compared to the responses from control sites. 100 % response was recorded from injection sites at 120 min which was similar to that recorded from control sites.

Effect of combination of bupivacaine and ketamine on cutaneous trunci muscle reflex (CTMR)

Table 3 shows the duration and percentage response after the administration of bupivacaine and ketamine combined in a single injection. Zero percent (0 %) response was recorded from injection sites during the first 40 min which was significantly different from the 100 % response recorded from control sites ($p < 0.05$). Minimal response was recorded at 60 and 100 min but these were still significantly different when compared to the responses from control sites. 100 % response was recorded from injection sites at 120 min which was not different from that recorded from control sites.

Effect of combined bupivacaine and epinephrine on cutaneous trunci muscle reflex (CTMR)

Table 4 shows the duration and percentage response after the administration of combined bupivacaine and adrenaline injection. Zero percent (0 %) response was recorded from injection sites for the first 120 min following injection and these were significantly different when compared to the responses from the control site. Minimal responses were recorded between 140 and 200 min but these were still significantly different from the responses recorded from the corresponding control site. 100 % responses were recorded from injection sites at 220 min with no significant difference when compared to responses from control sites.

Effect of combined ketamine and epinephrine on cutaneous trunci muscle reflex (CTMR)

Table 5 shows the duration and percentage response after the administration of combined ketamine and epinephrineine injection. Zero percent (0 %) response was recorded from injection sites for the first 100 min following injection and these were significantly different when compared to the responses from the control site. There was a progressive increase in the percentage of positive responses recorded between 100- and 200 min duration but these were still significantly different from the responses recorded from the corresponding control site. 100 % response was recorded from injection sites at 200 min with no significant difference when compared to responses from control sites.

Effect of combined bupivacaine, ketamine, and epinephrine on cutaneous trunci muscle reflex (CTMR)

The duration and corresponding percentage response to forceps bite after the injection of a combination of bupivacaine, ketamine, and epinephrine is represented in Table 6. From 0 min to 120 min, a 0 % response was recorded at the injection site. This was significantly different from the responses recorded at the control site during this same experimental period ($p < 0.05$). 28 ± 5 % and 76 ± 10 % responses were recorded at 140 min and 160 min respectively but these were still significantly different from the 100 % recorded at the corresponding control sites. At 180 min, a 100 % response was recorded at the injection site which was not different from the response recorded from control sites.

Table 2
Duration and corresponding percentage CTMR responses from control and injection sites following ketamine injection in female Wistar rats.

	Response (%)						
Duration (Mins)	0	20	40	60	80	100	120
Control	100±0	100±0	100±0	100±0	100±0	100±0	100±0
KET	0 ± 0*	0 ± 0*	0 ± 0*	28±15*	48±19*	72±12*	100±0

Values are expressed as the Mean ± Standard Error of Mean, $n = 5$,.
* $P < 0.05$ vs control, KET. = Ketamine.

Table 3
Duration and corresponding percentage CTMR responses on control and injection sites following injection of combined bupivacaine and ketamine in female Wistar rats.

	Response (%)						
Duration (Mins)	0	20	40	60	80	100	120
Control	100±0	100±0	100±0	100±0	100±0	100±0	100±0
BUP+KET	0 ± 0*	0 ± 0*	0 ± 0*	44±20*	56±16*	92±5*	100±0

Values are expressed as the Mean ± Standard Error of Mean, $n = 5$,.
* $P < 0.05$ vs control, BUP. = Bupivacaine; KET. = Ketamine.

Table 4
The effect of bupivacaine and epinephrine on cutaneous trunci muscle reflex in female Wistar rats.

	Response (%)											
Duration (Mins)	0	20	40	60	80	100	120	140	160	180	200	220
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
BUP+EPI	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	12 ± 5*	56 ± 7*	84 ± 1*	96 ± 4*	100 ± 0

Values are expressed as the Mean ± Standard Error of Mean, $n = 5$,.
* $p < 0.005$ vs control site, BUP = Bupivacaine; EPI = Epinephrine.

Table 5
Effect of co-administration of ketamine and epinephrine on cutaneous trunci muscle reflex in female Wistar rats.

	Response (%)										
Duration (Mins)	0	20	40	60	80	100	120	140	160	180	200
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
KET+EPI	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	20 ± 20*	20 ± 20*	52 ± 14*	68 ± 8*	100 ± 0

Values are presented as mean ± Standard Error of Mean, $n = 5$.
* $p < 0.05$, KET. = Ketamine; EPI. = Epinephrine.

Table 6
Combined effect of ketamine, bupivacaine and epinephrine on cutaneous trunci muscle reflex in female Wistar rats.

	Response (%)									
Duration (Mins)	0	20	40	60	80	100	120	140	160	180
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
BUP+KET+EPI	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	28 ± 5*	76 ± 10*	100 ± 0

Values are expressed as Mean ± Standard Error of Mean, n=5,.
* $p < 0.005$ vs control, BUP. = Bupivacaine; KET. = Ketamine; EPI. = Epinephrine.

Duration at first observable positive CTMR at injection sites

Fig. 1 shows the time at which the first positive CTMR response was observed after the administration of bupivacaine alone, ketamine alone, and combined administration of bupivacaine and ketamine with and without epinephrine. The first observable positive CTMR at the injection sites was recorded at 120 min, 60 min, and 60 min respectively for bupivacaine alone, ketamine alone, and combined bupivacaine and ketamine injections (Fig. 1A). However, the duration at the time of first observed positive response increased to 140 min, 120 min and 140 min for bupivacaine plus epinephrine, ketamine plus epinephrine and combined bupivacaine, ketamine and epinephrine injections respectively (Fig. 1B).

Duration at the time of complete recovery of pain sensation (100 % positive CTMR) at injection sites

Fig. 2 shows the duration at the time when the complete recovery of pain sensation was observed at the injection sites. 100 % positive CTMR was recorded after 160 min, 120 min, and 120 min of the administration of bupivacaine alone, ketamine alone, and combined bupivacaine and ketamine respectively (Fig. 2A). Similarly, the duration at the time of 100 % positive response was 220 min, 200 min, and 180 min for bupivacaine plus epinephrine, ketamine plus epinephrine, and combined bupivacaine, ketamine, and epinephrine injections respectively (Fig. 2B).

Discussion

Bupivacaine as demonstrated in this study was found to block cutaneous trunci muscle reflex for about 120 min (2 h). This duration

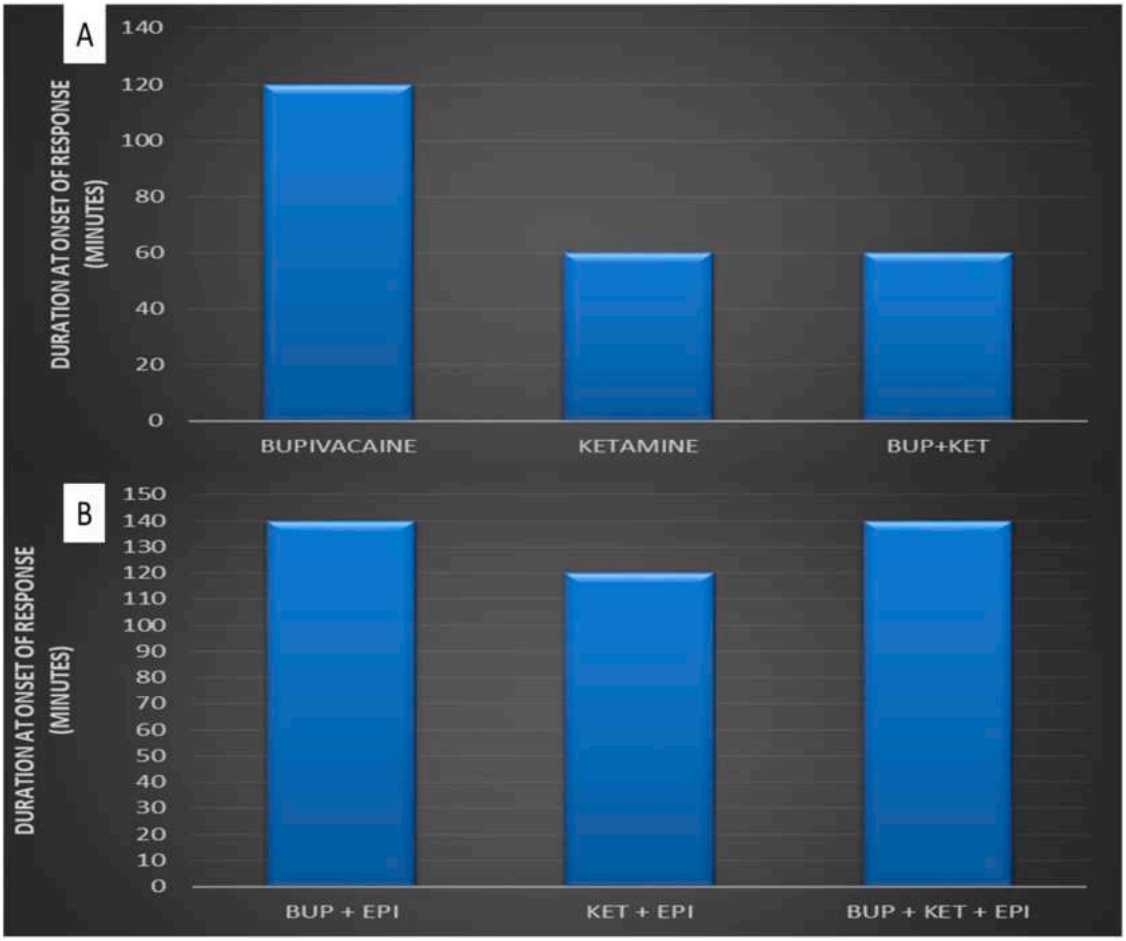


Fig. 1. Bar chart showing durations at the time of first observed positive CTMR after the injection of Bupivacaine alone, Ketamine alone and a combination of both bupivacaine and ketamine, without epinephrine (A) and with Epinephrine (B).

of analgesia appears to be within the range reported by Liu et al. [8] that bupivacaine when administered subcutaneously is expected to act for a duration of between 2 and 4 h. The duration of induced analgesia for locally infiltrated bupivacaine is however largely dependent on the volume and concentration of drug administered. A study carried out to access the time-course profile and bupivacaine concentrations at the site of injection following subcutaneous administration reported that a single dose of standard bupivacaine may last for approximately 8 h [9]. This is a clear demonstration of the role played by volume and concentration on the duration of analgesia induced by locally infiltrated bupivacaine. Bupivacaine provides analgesia through the blockade of voltage-gated sodium channels, thereby preventing the activation of sensory pain pathways and central sensitization.

The duration of analgesia induced by ketamine as demonstrated in this study was shorter when compared to that of bupivacaine. Bupivacaine has been reported to have a slower onset of action but a longer duration of action compared to ketamine [10]. Additionally, ketamine has a lower affinity for transport proteins thereby moving freer in circulation and thus easier metabolized. Ketamine inhibits pain by binding to N- methyl D Aspartate receptor (NMDA), which might then inhibit glutamate-induced activation of NMDA receptors on primary afferents in the skin, which may consequently decrease peripheral nociceptive input into the spinal cord and central sensitization of the dorsal horn. This is the mechanism of the local analgesia induced by Ketamine. Another rather interesting finding in this study is the observation of a shorter duration of analgesia induced when ketamine was co-administered with bupivacaine as compared to the duration induced by either of the two drugs individually This appears to corroborate the report of Yanli et al. [11] and Weir et al. [12] that ketamine combined with other local anesthetics do not produce any improved analgesic effects. Similarly, in a study by Togal et al. [13] ketamine administered concurrently with low-dose bupivacaine provided shorter motor and sensory block onset time, shorter duration of action, and less motor blockade, which resulted in less duration compared to when Ketamine and Bupivacaine were administered separately [13].

It is important to reiterate that the volume of drug infiltrated in this present study for all groups remained the same, 0.6 mls, which invariably implies a lower volume of each of the component drugs for the groups co-administered with two or more drugs. Since the local cutaneous analgesic effects of bupivacaine and ketamine are said to be permissive but not additive, the total duration observed after co-administration of both drugs therefore represents the duration offered by the reduced volume of each drug when given

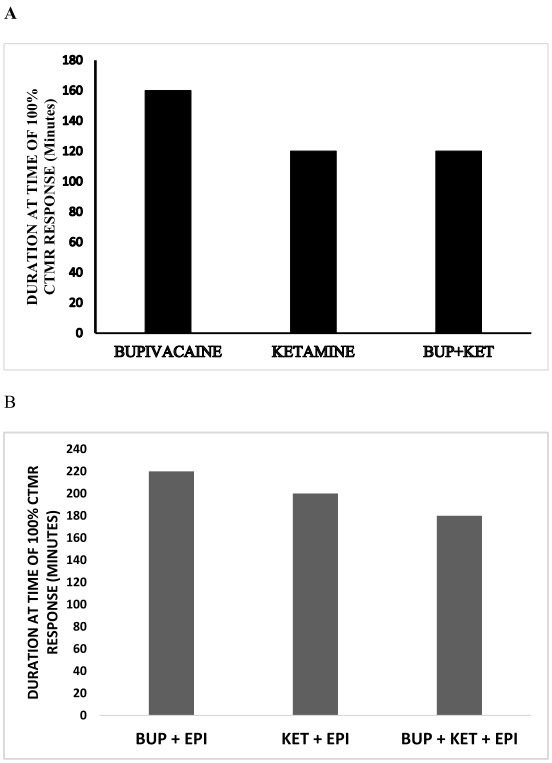


Fig. 2. Bar chart showing durations at the time at which 100 % positive CTMR was recorded after the injection of Bupivacaine alone, Ketamine alone and a combination of both bupivacaine and ketamine without epinephrine (A) and with epinephrine (B).

separately. However, according to Zhag et al. [14], the analgesic effect of ketamine and bupivacaine is synergistic when co-administered intrathecally, and a longer duration of action has been reported for the combination when compared to individual injections of both drugs via intrathecal route. This finding implies that apart from volume and concentration, the route of administration of analgesic agents may be an important key factor in determining the duration of action.

The co-administration of both bupivacaine and ketamine with epinephrine either individually or as a combination of both drugs significantly prolonged the duration of analgesia produced. For over a century, epinephrine has been added to local anaesthetic/analgesic solutions to delay the absorption of the drug and to prolong and enhance its analgesic effect. Shyamala et al. [15] compared the effectiveness of bupivacaine with or without epinephrine and reported that the combination of bupivacaine and epinephrine prolonged the duration of action compared to bupivacaine alone. This effect has been attributed to the epinephrine-mediated local vasoconstriction that decreases local blood flow, thereby resulting in slower anaesthetic clearance from the injection site [16]. Several studies have demonstrated a decrease in peak plasma concentrations of local anaesthetics when they are co-administered with epinephrine for both peripheral and central blockades [8,17]. This, as well as the reduced blood flow caused by epinephrine results in slower metabolism of the analgesics and ultimately longer duration of action.

Conclusion

With the findings reported in this study, it was concluded that the combination of ketamine and bupivacaine for use as local analgesia is not proficient as long as the aim is to enhance the duration of analgesia. This may be because the interaction between the two drugs is permissive rather than additive. However, the concurrent administration of each or both drugs with epinephrine results in a significant prolongation of the duration of analgesia induced.

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CRediT authorship contribution statement

Dennis Seyi Arokoyo: Conceptualization, Visualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Praise Ireyimika Fagbangbe:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Oluwabanke Chigozirim Oladimeji:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Abraham Oluwaseun Olaoye:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Ayobami Emmanuel Oyediran:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Majeed Babajide Adegboye:** Conceptualization, Visualization, Methodology, Data curation, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest with regards to this work.

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