

Evaluation of antinociceptive activity of ketamine cream in rats

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Abstract. Objective: To examine the analgesic and antinociceptive effect of ketamine cream in rats as an animal model. Materials and methods: Fifteen healthy adult albino rats with equal age and gender distribution of either sex were selected for this study. The animals were divided into 3 groups of 5 animals. The pain reaction time was recorded pretreatment for each animal and was taken as a basal threshold (Tayebi et al., 2008). Group 1 served as a control and was applied cream topically on fore and hind limb. Group 2 and 3 were applied topically ketamine cream 2.5%, 5% respectively on fore and hind limb. The onset and duration of analgesic effect of ketamine cream were evaluated in rats by utilizing a Hot-Plate test at $55 \pm 1^\circ\text{C}$. Latency reaction time was recorded after 3min. and (10, 20, 30, 40, 50, 60 min.) The prolongation of latency times compared with the values of the control was used to express about antinociceptive effects of ketamine cream and the percentage of antinociceptive Maximal Possible Effect (MPE) was calculated. Results: demonstrated that the ketamine cream at concentrations (2.5, 5) % produced antinociceptive effect in rats (11.0 ± 1.4)(16.5 ± 9.0) second after 3 min, respectively in comparison with control (7.0 ± 0.8) second. The percentage of maximum possible effect (MPE) was increased from (3.16) % in control group to (21.65), (42.5)% respectively according to the above concentrations of ketamine cream after 3 min. The duration of analgesia with topical application of cream (2.5, 5)% from 3-40 min and usually approached the base line (5.75 ± 1.5) (7.0 ± 1.4) second respectively after 60 min. Conclusions: This study concluded that ketamine cream have a good antinociceptive activity in rats after 3 minutes of topical application and prolong the duration of analgesia more than 40 minutes depending on concentration of ketamine cream.

Key Words: Ketamine cream, pain, hot-plate test, antinociceptive, maximal possible effect.

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Introduction

Ketamine is classified as an NMDA receptor antagonist (Blonk et al 2010), it is used for pain treatment. The systemic administration of ketamine is less used due to the side effects such as hallucinations, nausea and vomiting (Cleane 2000).

Analgesics drugs are commonly prescribed for pain management (Curtero et al 2006). The analgesic drugs can be defined as a drug that relieves, manages and treat pain (Dahl and Reader 2000). Systemic administration of analgesic drug produces many side effects. Therefore it is of paramount importance to develop non-oral dosage forms of analgesic drug to minimize the gastric side effects, to persist for a long period at the site of application and to overcome the disadvantages of oral administration (Alarcon 2002). Given that the topical applied drug is considered the best route of administration because by passing through the skin, it has a large area for drug penetration with fast exposure to circulating and the route is noninvasive (Daniels and Kine 2007).

Ketamine, has been used for more than 30 years to produce anesthesia (Orser et al 1997). It is not usually used for treatment of humans, because it produce several side effects such as hallucinations, nightmares, dissociative reactions. Patients become addicted to ketamine after a long period of administration when given orally or intravenously (Peck et al, 2008, Yagiela et al 2011). Nowadays studies regarding ketamine found it has an analgesic activity as well as anesthetic effect. Although the mechanisms

of ketamine's analgesic effects remains not clear and are likely multiple (Meller 1996). Ketamine is an antagonist of N-methyl 1-D-Aspartate (NMDA) class of glutamate receptors which is largely responsible for its anesthetic and behavioral effects (Yagiela et al 2011). Ketamine is used as a general anesthesia, usually in combination with a sedative and can be also used in small doses as an analgesic, particularly for the treatment of pain associated with movement, neuropathic pain, and to relieve acute pain (Annetta et al 2005). The topical ketamine administered to patients with chronic neuropathic pain is effective to reduce allodynia and hyperalgesia (Epstein et al 2001). Therefore, the aim of the present study is to prepare different concentration of ketamine cream to examine the analgesic and antinociceptive effects of topical application in rats by using a Hot plate test.

Material and Methods

Animals

Fifteen healthy adult albino rats with equal age and gender distribution of either sex weighing 200-300 gm were selected to use for this study. Rats were obtained from animal care housed in Dentistry Collage of Mosul University in Iraq. The animals were housed in plastic cages under 12h light\12h dark cycle at $22 \pm 2^\circ\text{C}$ and access to fed with standard diet and tap water ad libitum.

Preparation of ketamine cream

Ketamine cream were prepared in two different concentration by mixing (2.5, 5) g from ketamine powder in 100 g cold cream to give final concentration (2.5%, 5%) W\W with continuous mixing using a glass rode until homogenous cream were formed. The cream were kept in plastic containers and stored in refrigerator at 40C until used.

Experiment on animals

The rats were divided into 3 groups; each group consisted from five animals. The pain reaction time was recorded pretreatment for each animal and was taken as a basal threshold. Group 1 served as a control and was applied only cream topically on fore and hind limb. Group 2 and 3 were applied ketamine cream 2.5%, 5% respectively topically on fore and hind limb. The onset and duration of analgesic effect of ketamine cream were evaluated in rats by utilizing a Hot-Plate test. Rats were placed on a hot-plate maintained at $55\pm 1^{\circ}\text{C}$. The reaction time is that between placing the animals on the hot-plate and holding, jumping, licking of the fore or hind paws. Acute off time of 30 seconds is followed to avoid any thermal injury to the paws (Ghosh1984). Latency reaction time was recorded after 3 min. and (10, 20, 30, 40, 50, 60 min.) following topically applied of ketamine cream in treated group and cream only in control group by using hot-plate test to assess the onset and duration of treated cream. The prolongation of latency times compared with the values of the control was used to express about antinociceptive effects of ketamine cream.

The percentage of antinociceptive Maximal Possible Effect (MPE) was calculated from the formula: (Giusti et al 1997)
 $\%MPE = (\text{Test latency} - \text{pre drug latency} / \text{cut off time} - \text{pre drug latency}) \times 100$; MPE: Percentage of antinociception maximal possible effect. Test latency: Sec after drug treatment. Pre drug latency: Sec before drug treatment at zero time. Cut off time: 30 second.

Statistical analysis

The data were expressed as mean \pm SD, difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at $p < 0.05$.

Results

In the present study, the ketamine cream was evaluated for its antinociceptive activity against pain induced by hot-plate in rats. Topical application of ketamine cream at (2.5, 5) % produce good analgesic effects in comparison with control group treated by cream only.

The results of assessment of the increase in pain reaction time of ketamine cream at (2.5)% and (5)% shown that the cream has a highly significant difference after (3 and 10, 20, 30, 40, 50, 60) min from application of cream alone (Table 1).

Topical application of ketamine at (2.5)% lead to significant increase in pain threshold between pre and post treatment (Table, 2). While topical application of ketamine at (5)% lead to significant increase in pain threshold between pre and post treatment (Table 2), whereas in control group application of cream alone no significantly difference between pre and post treatment (Table 2).

Reaction time (antinociceptive(MPE)) was increased from (3.16)% in control treated group after 3min to (21.65, 42.5)% respectively according to the concentration of ketamine cream at (2.5, 5)% (Figure 1).

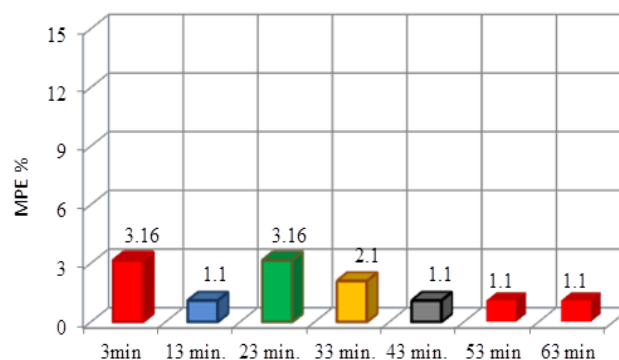


Fig. 1. Effect of cream alone on antinociceptive maximal possible effect (MPE) in hot plate test in rats

In the hot plate test, the percentage of reaction time (antinociceptive) in concentration of ketamine at 2.5% increased according to the time (Figure 2) in comparison to control group (Figure 1).

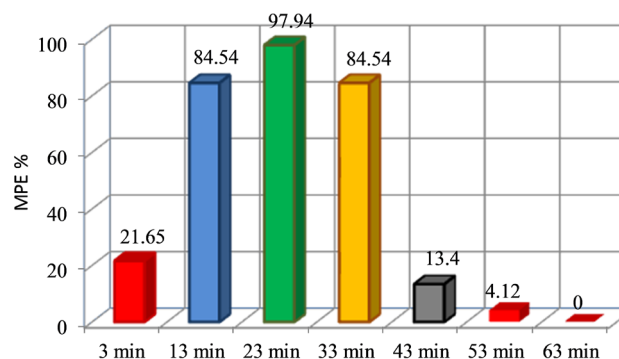


Fig. 2. Effect of ketamine 2.5% on antinociceptive maximum possible effect (MPE) in hot plate test in rats

While in concentration of ketamine cream at 5% the (MPE) increased to (42.5, 94.68, 98.92, 50, 20.21, 2.13, 2.13)%. (Figure 3).

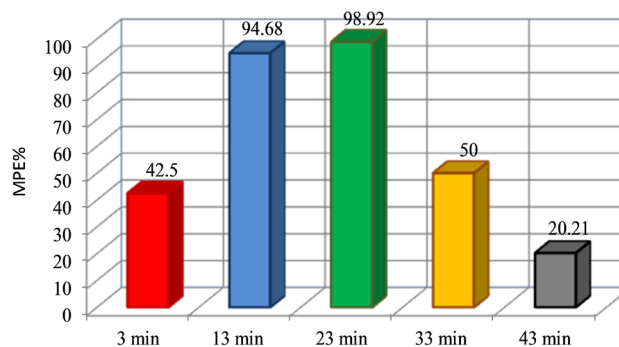


Fig. 3. Effect of ketamine cream (5%) on antinociceptive maximum possible effect (MPE) in hot plate test in rats

Table 1. The pain reaction time of ketamine cream in rats

Conc.	Time							
	+3 Min.	+10 Min.	+20 Min.	+30 Min.	+40 Min.	+50 Min.	+60 Min.	
Cream alone (Control)	7.0±0.8	6.5±1.0	7.0±0.8	6.75±0.5	6.5±0.5	6.5. ±0.5	6.5. ±0.5	
Ketamine Cream 2.5%	11.0±1.4	26.25±3.3*	29.5±1.0*	26.25±2.8*	9.0±2.4	6.75±1.5	5.75±1.5	
Ketamine Cream 5%	16.5±9.0*	28.75±2.5*	29.5±1.0*	18.25±6.0*a	11.25±4.7	7.0±1.4	7.0±1.4	

Values are expressed as mean ±SD; * significant with control at $p \leq 0.05$; a: significant with ketamine cream 2.5 % at $p \leq 0.05$.

Table 2. The increase in pain reaction time threshold of ketamine cream in rats

Conc.	Time							
	+3 min	+10 min	+20 min	+30 min	+40 min	+50 min	+60 min	
Cream alone (Control)	0.75±0.9	0.75±0.9	1.0±0.2	0.5±0.5	0.25±0.1	0.25±0.5	0.25±0.5	
Ketamine Cream 2.5%	5.5±2.3	20.5±5.4 *	23.75±0.84*	20.5±3.1*	3.25±2.91	1.0±0.8	1.0±0.8	
Ketamine Cream 5%	10.0±8.6 *	22.25±2.2*	23.0±1 *	11.75±1.4*a	4.75±4.5	0.5±1.0	0.5±1.0	

Values are expressed as mean ±SD; * significant with control at $p \leq 0.05$; a: significant with ketamine cream 2.5 % at $p \leq 0.05$.

The maximum effect (best time) action of ketamine cream always happen at 20 min. in different concentration (2.5, 5)% (Figure 4). The mean ± SD of duration of analgesia obtained with topical application of cream (2.5, 5)% from 3-40 min and usually approached the base line (5.75±1.5) (7.0±1.4) respectively after 60 min (Figure 4).

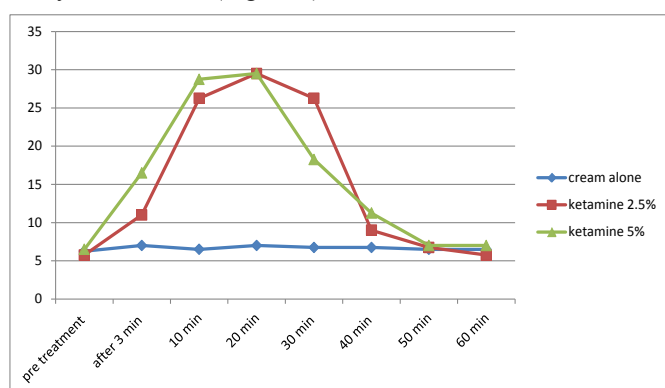


Fig. 4. Duration of analgesic activity of ketamine cream (2.5 and 5) % after topical application.

Discussion

Topical analgesics drugs application have many advantages such as the ability to provide good analgesia with reduced systemic drug levels (Kreitler and Niv 2007). Long period remain at the application site and enough drug penetration and high effective are other advantages (Illum et al 1994). Ketamine has central and peripheral mechanism; the analgesic efficiency of ketamine cream in the present study may be due to its action on NMDA receptors (Yagiela et al 2011). The NMDA receptor is a receptor that allows for the transfer of electrical signals between neurons in the brain and in the spinal column. For electrical signals to pass, the NMDA receptor must be open. To remain open, glutamate and glycine must bind to the NMDA receptor as an antagonists (Kim et al 2002).

In the present study the analgesic properties of ketamine cream were observed on rats by using a hot - plate test. The obtained results are in line with previous study that suggested that the application of topical ketamine gel produce antinociceptive effect in human and animals at 0.5% (Alhussary 2013; Akassaz 2014). The present study has demonstrated the antinociceptive effect of topical ketamine cream begins after 3 minutes. The concentration of ketamine (2.5, 5)% have significantly increased the response latency time at $p < 0.05$. This could be the possible explanation for its central analgesic activity observed in hot plate test because NMDA receptor antagonism affects analgesia by preventing central sensitization in dorsal horn neurons, these neurons are important pharmacological site of action for the antinociceptive effects of different drugs during spinal anesthesia. In other words, ketamine's actions interfere with pain transmission in the spinal cord (Quibell et al 2011). Another mechanism of action may be due to the fact that ketamine inhibits nitric oxide synthase, inhibiting production of nitric oxide, a neurotransmitter involved in pain perception, and hence further contributing to analgesia (Aroni et al 2009).

The activation of (NMDA) receptors caused pain, this activation can be prevented by agents that block the effects of glutamate on NMDA receptor (Lauretti et al 1999). Therefore NMDA receptor antagonist are used in the treatment of neurogenic and sever pain stages (Sang et al 2002). Another possible explanation of the analgesic activity of ketamine cream, ketamine acts on opioid receptors (Epstein et al 2001). It interacts with sigma and opioid μ receptors to relieve pain. Ketamine has affinity to many receptor types, including opioid Mu and kappa receptors (Narita et al 2001; Rowland et al 2005). Therefore the present study, is in line with previous reports that underline that ketamine produced antinociceptive effect due to both endogenous and exogenous opioids and can also produce opioid- mediated analgesia at sites outside the CNS. Ketamine has also other mechanism of action by the effects of ketamine on ion channels. It is demonstrated that ketamine blocks peripheral and

central nervous system Na⁺ channels (Scheller et al 1996), voltage sensitive K⁺ (Braw et al 1997), and voltage-gated calcium channels (Rossi 2011). In the last years researchers demonstrated that ketamine inhibits the neuronal reuptake of nor-epinephrine and serotonin to produces antinociceptive effects (Natalini et al 2007).

Conclusion

Topical ketamine cream is an alternative dosage form, it is easy to apply, safe and not irritant. Because of the ketamine having all these above mechanisms, our study showed that ketamine cream have a good analgesic effect on the pain stimulated by the hot-plate when using it in the rats.

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