

KETAMINE INDUCED ANALGESIA IN MICE AT SUB-PSYCHOTOMIMETIC DOSE

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SUMMARY

The roles of N- Methyl –D- Aspartate receptor in the processing of nociception has led to renewed clinical interest in ketamine, an antagonist of NMDA receptor. Low-dose ketamine had been reported to possess analgesic effect though the paucity and inconsistency of such data have called for direct evaluation of this claim. This study therefore explored the analgesic effect of a sub-psychotomimetic dose of ketamine (SPDK), evaluated such effect on morphine- and diclofenac-induced analgesia and determined its possible neuronal mechanism of analgesia. Mice weighing between 18-25 g were randomly distributed into two major groups consisting of Group 1 and 2 which were used for the assessment of analgesic effect and determination of neuronal mechanism of 1 mg/kg ketamine using the hot plate model; and the formalin-induced pain model respectively. Data were presented as mean $\pm$ standard error of mean SEM and analyzed using ANOVA followed by post-hoc analysis (Student-Newman-Keuls) and $P < 0.05$ was set as an accepted level of significance. The SPDK induced significant analgesia in the hot plate model but caused allodynia in the formalin-induced pain model. In addition, SPDK potentiated morphine-induced and diclofenac-induced analgesia in both the hot plate and formalin tests, while naloxone significantly blocked its analgesic effect at 90 minutes post-administration in the hot plate test. This study showed that SPDK induced analgesia in the acute pain model but aggravated pain in the chronic model. It also potentiated both morphine and diclofenac-induced analgesia possibly mediated through modulation of opioidergic pathway in mice.

Key words: Sub-Psychotomimetic Dose, Ketamine, morphine, diclofenac, allodynia, hot plate model, formalin-induced pain model, NMDA receptors, opioidergic pathway

Résumé

Les rôles du récepteur N-Méthyl-D-Aspartate dans le traitement de la nociception ont suscité un regain d'intérêt clinique pour la kétamine, un antagoniste du récepteur NMDA. La kétamine à faible dose aurait eu un effet analgésique, bien que la rareté et le manque de cohérence de ces données appellent une évaluation directe de cette affirmation. Cette étude a donc exploré l'effet analgésique d'une dose sous-psychotomimétique de kétamine (SPDK), évalué cet effet sur l'analgésie induite par la morphine et le diclofénac et déterminé son mécanisme neuronal potentiel d'analgésie. Des souris pesant entre 18 et 25 g ont été réparties de manière aléatoire dans deux groupes principaux composés des groupes 1 et 2, qui ont été utilisés pour évaluer l'effet analgésique et déterminer le mécanisme neuronal de 1 mg / kg de kétamine à l'aide du modèle à plaque chauffante; et le modèle de douleur induite par la formaline respectivement. Les données ont été présentées en tant que moyenne $\pm$ erreur type de l'ESM moyenne et analysées à l'aide d'une ANOVA suivie d'une analyse post-hoc (Student-Newman-Keuls) et $p < 0,05$ a été défini comme un niveau de signification accepté. La SPDK a induit une analgésie significative dans le modèle de la plaque chauffante, mais a provoqué une allodynie dans le modèle de la douleur induite par le formol. En outre, SPDK a potentialisé l'analgésie induite par la morphine et par le diclofénac dans les tests à la plaque chauffante et au formol, tandis que la naloxone a significativement bloqué son effet analgésique 90 minutes après l'administration dans le test à la plaque chauffante. Cette étude a montré que l'analgésie induite par la SPDK dans le modèle de douleur aiguë, mais aggravait la douleur dans le modèle chronique. Il a également potentialisé l'analgésie induite par la morphine et le diclofénac, éventuellement induite par la modulation de la voie opioidergique chez la souris.

Mots-clés: Dose sous-psychotomimétique, Kétamine, morphine, diclofénac, allodynie, modèle de plaque chauffante, modèle de douleur induite par le formol, récepteurs NMDA, voie opioïde

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INTRODUCTION

Almost all pathological conditions known in man are accompanied with pain. Pain is the most common symptom that makes patients visit healthcare practitioners especially in low-resource countries (Soyannwo, 2010). The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey, 1991). It is often caused by intense or damaging stimuli or sometimes in the absence of any detectable stimulus, damage or pathology. Biologically, pain is believed to be part of the body's defense system (Melzack *et al.*, 1996), thus considered as a complex sensory modality essential for survival (Patel, 2010). Since pain is a major symptom in many medical conditions and as such interferes with person's quality of life and general functioning, gainful access to pain relief and management is therefore greatly required.

Despite the fact that several analgesic agents are available for the treatment of virtually all kinds of pain, the presence of wide range of side effects like gastric lesions (Lawal, 2013) caused by irrational use of non-narcotic analgesics (Olaleye, 2013) on the one hand; and tolerance and dependence induced by opiates on the other hand, have limited their therapeutic values (Kumara, 2001). Similarly, erratic or non-availability and inaccessibility of opioid analgesics caused by administrative bottlenecks in their distribution especially in the developing societies (Folorunso, 2013); and a fear of using opioids because of an erroneous belief that their use will inevitably cause addiction (Soyannwo, 2010) have significantly reduced gainful access to pain relief. The relief of pain was published to be a human right following the launch of the first Global Campaign Against Pain in Geneva in 2004 by the International Association of Pain (IASP), the European Federation of Chapters of the IASP (EFIC), and the World Health Organization (WHO, 2000) yet, a large number of patients suffering from pain especially, in developing countries do not receive adequate treatment for pain.

Excitatory Amino Acid (EAA) receptors to which NMDA antagonists e.g. ketamine, block are known to be involved in the spread of pain beyond its origin of stimulation via a phenomenon called *long term potentiation* (LTP) (Okpako, 2002). This involvement of N-Methyl-D-Aspartate (NMDA) receptor in the processing of nociception has therefore led to renewed clinical interest in ketamine (Petrenko *et al.*, 2003). Ketamine is a potent dissociative anaesthetic agent and had been reported to possess analgesic effect even at sub-anaesthetic doses (Aroni *et al.*, 2009; Bell, 2009; Borsook, 2009; Bell and Moore, 2010; Qutaiba *et al.*, 2013; Owolabi *et al.*, 2018). However, the presence of psychotomimetic adverse effects had discouraged its use in pain management. It is a piperidine derivative with a unique ability to safely provide anxiolysis, analgesia, and amnesia simultaneously (McCarthy *et*

al., 2013). It is water and lipid soluble, rapidly crosses the blood brain barrier and has a short duration of action (Clement and Nimmo, 1981 and White *et al.*, 1982). Ketamine acts by having a direct effect on the cortex and limbic system and/or decreasing muscle spasms via NMDA receptor antagonism (Lodge *et al.*, 1982 and Alex, 2014).

Since reports on sub-anaesthetic doses of ketamine-induced analgesia are very few and inconsistent; and another segment of this research work (Atunwa, 2015) has considered 1 mg/kg ketamine as a sub-psychotomimetic dose of ketamine (SPDK), this study therefore explored possible analgesic effect of the sub-psychotomimetic dose of ketamine; evaluated such effect on morphine-induced and diclofenac-induced analgesia using acute and chronic pain models in mice; and determined its neural mechanism of analgesia with an overall view to providing information on the possibility of its application in pain management.

MATERIALS AND METHODS

Animals

Eighty-four Swiss albino mice of either sex weighing 18-25 g were used for this study. The mice were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University (O. A. U.), Ile-Ife, Osun State, Nigeria. They were housed in the animal house of the Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife. This was followed by random distribution to different groups of six-member each. The animals had access to food and water *ad libitum*. Ethical approval for the study (PHP12/13/H/0607) was obtained in 2015 from the Research Ethics Committee of O. A. U. Postgraduate College and guidelines applying to animal use and experimental procedures as stipulated by the committee were duly observed. All experimental procedures were conducted between 8 a.m. and 6 p.m.

Drugs and Reagents

Ketamine hydrochloride (50 mg/ml, Rotex Medica Trittau, Germany), Diclofenac potassium (75 mg/3ml, Embassy Pharma & Chem. Ltd, Nigeria), Morphine hydrochloride 10 mg/kg, Naloxone hydrochloride 1 mg/kg (Sigma and St Louis, MO, USA), Normal saline (Drugfield Pharmaceuticals Ltd, Nigeria), Methylated spirit (Royal Priesthood Lab. Ltd, Nigeria), Formalin (2.5%) and Distilled water (freshly prepared at Pharmacology Laboratory, O.A.U., Ile-Ife). All reagents and drugs were stored according to the manufacturers' recommended storage conditions.

Experimental Procedures

Hot Plate Test (Acute Pain Model)

Twenty-four mice were randomized into four groups ($n = 6$) and used in assessing the analgesic effect of sub-psychotomimetic dose of ketamine (1 mg/kg). Groups 1, 2, 3 and 4 received 10 μ l/g normal

saline, 1 mg/kg ketamine, 75 mg/kg diclofenac (Woode *et al.*, 2008), and 10 mg/kg morphine, intraperitoneally (Akanmu *et al.*, 2011) respectively. Thereafter, each mouse was placed on the hotplate which has been pre-set at a temperature of $55^{\circ}\text{C} \pm 0.5$ (Vogel *et al.*, 2002) at 5, 20, 60, and 90 minutes post-treatment to determine its pain threshold as described by Almeida *et al.*, 2008. A cut-off time of 25 seconds was set to avoid tissue damage in mice (Ratnasooriya *et al.*, 2003).

Another set of mice were distributed into two groups ($n = 6$) and used in evaluating the effects of 1 mg/kg ketamine on morphine and diclofenac-induced analgesia. Ketamine 1 mg/kg was concomitantly administered each along with 75 mg/kg diclofenac and 10 mg/kg morphine (Javery *et al.*, 1996) i.p. to groups 1 and 2 respectively. Thereafter, they were subjected to hot plate test. Twelve mice were allocated into another two groups ($n = 6$) and used in the determination of possible neural mechanism of action of ketamine-induced analgesia. Mice were pre-treated with 1 mg/kg naloxone (s.c.) fifteen minutes (Akanmu *et al.*, 2011) before the administration of 10 μg /g normal saline or 1 mg/kg ketamine i.p. into group 1 and 2 respectively. Thereafter, they were subjected to hot plate test.

Formalin-Induced Pain Test (Chronic Pain Model)

Twenty-four mice were distributed into four groups ($n = 6$) and used in assessing the analgesic effect of sub-psychotomimetic dose of ketamine (1 mg/kg). Groups 1, 2, 3 and 4 received 10 μg /g normal saline, 1 mg/kg ketamine, 75 mg/kg diclofenac (Woode *et al.*, 2008), and 10 mg/kg morphine i.p. (Akanmu *et al.*, 2011) respectively. After 5 minutes of drug treatment, each mouse was injected with 0.02 ml of 2.5 % formalin solution (0.92% formaldehyde diluted in saline) into the sub-plantar region of its hind paw (Murray *et al.*, 1988 and Almeida *et al.*, 2008) and immediately placed in a transparent plastic cage for observation. Evaluation was made as described by Hunskaar *et al.*, 1985; and Sawynok and Liu, 2003.

Another set of mice were allotted into two groups ($n = 6$) and used in evaluating the effects of 1 mg/kg ketamine on morphine and diclofenac-induced analgesia. 1 mg/kg ketamine was concomitantly administered each along with 75 mg/kg diclofenac and 10 mg/kg morphine (Javery *et al.*, 1996) i.p. to groups 1 and 2 respectively. Thereafter, they were subjected to the formalin-induced pain test.

Statistical Analysis

Data were expressed as Mean $\pm$ standard error of mean (SEM). The results were evaluated using One-way Analysis of Variance (ANOVA), followed by post-hoc test (Student-Newman-Keuls Multiple Comparisons Test). $p < 0.05$ was considered as accepted level of significant difference from the controls. GraphPad InStat® Biostatistics software (GraphPad Software Inc., La Jolla, USA) was used for data analysis.

RESULTS

Hot Plate Test

Post-hoc analysis showed that the sub-psychotomimetic dose of ketamine (1 mg/kg) elicited ketamine-induced analgesia at 90 minutes ($F_{(3, 21)} = 3.955$, $p < 0.05$) post-administration but rather, allodynic effect was observed at 60 minutes post-administration as presented in Table I. However, ketamine-induced analgesia was potentiated at 5 minutes ($F_{(6, 35)} = 8.216$, $p < 0.001$) and 60 minutes ($F_{(6, 35)} = 5.491$, $p < 0.01$) post-treatment when co-administered with morphine 10 mg/kg; and diclofenac 75 mg/kg respectively whereas, ketamine-induced allodynia were observed at 60 minutes ($F_{(6, 35)} = 7.308$, $p < 0.001$) and 90 minutes ($F_{(6, 35)} = 5.161$, $p < 0.01$) post-treatment when co-administered with morphine 10 mg/kg; and diclofenac 75 mg/kg respectively as shown in Table II. Expectedly, 10 mg/kg morphine showed significant analgesia when compared to saline groups as depicted in Table 1. Meanwhile, naloxone significantly blocked ketamine-induced analgesia at 90 minutes ($F_{(5, 30)} = 6.731$, $p < 0.001$) post-treatment when evaluated using the hotplate test as shown in Figure 1.

Table 1: Assessment of Analgesic Effect of Sub-Psychotomimetic Dose of Ketamine 1 mg/kg at 5, 20, 60, and 90 Minutes Post-Treatment Using Hot Plate Method in Mice ($n = 6$)

TREATMENT	LATENCY OF PAW LICK (seconds)			
	5 minutes	20 minutes	60 minutes	90 minutes
SALINE 10 $\mu\text{g}/\text{kg}$	4.15 $\pm$ 0.20	6.80 $\pm$ 1.60	9.33 $\pm$ 1.20	6.67 $\pm$ 0.94
KETAMINE 1 mg/kg	3.22 $\pm$ 0.19	3.80 $\pm$ 0.61	3.47 $\pm$ 0.31*	9.77 $\pm$ 0.97*
DICLOFENAC 75 mg/kg	4.73 $\pm$ 0.51	7.47 $\pm$ 0.67	5.50 $\pm$ 0.56	13.33 $\pm$ 0.68
MORPHINE 10 mg/kg	7.22 $\pm$ 1.15	18.4 $\pm$ 1.95	21.7 $\pm$ 1.97	7.96 $\pm$ 0.45

Data presented as mean $\pm$ SEM; * $p < 0.05$ significantly different from saline group.

Table II: Evaluation of the Effect of Sub-Psychotomimetic Dose of Ketamine (1 mg/kg) on Morphine and Diclofenac-Induced Analgesia at 5, 20, 60 and 90 Minutes Post-Treatment Using Hot Plate Method in Mice ($n = 6$)

TREATMENT	LATENCY OF PAW LICK (seconds)			
	5 minutes	20 minutes	60 minutes	90 minutes
KETAMINE 1 mg/kg	3.22 $\pm$ 0.19	3.80 $\pm$ 0.61	3.47 $\pm$ 0.31	9.77 $\pm$ 0.97
DICLOFENAC 75 mg/kg	4.73 $\pm$ 0.51	7.47 $\pm$ 0.67	5.50 $\pm$ 0.56	13.33 $\pm$ 0.68
DICLOFENAC 75 mg/kg + KETAMINE 1 mg/kg	8.47 $\pm$ 1.51	11.85 $\pm$ 2.46	12.35 $\pm$ 1.98**	8.48 $\pm$ 1.45**
MORPHINE 10 mg/kg	7.22 $\pm$ 1.15	18.4 $\pm$ 1.95	21.7 $\pm$ 1.97	7.96 $\pm$ 0.45
MORPHINE 10 mg/kg + KETAMINE 1 mg/kg	16.68 $\pm$ 1.99*	21.1 $\pm$ 2.86	12.58 $\pm$ 1.21*	8.65 $\pm$ 0.56

Data presented as mean $\pm$ SEM; * $P < 0.05$ significantly different from morphine group,

** $p < 0.05$ significantly different from diclofenac group

Formalin Test

On the other hand, ketamine 1 mg/kg did not induce analgesia but rather allodynia in mice at both early (0-5 minutes) and late (15-30 minutes) phases in the duration and frequency of paw-lick using the formalin-induced pain model as presented in Table 3. However, the concomitant administration of 10 mg/kg morphine and 1 mg/kg ketamine significantly potentiated ketamine-induced analgesia at the early rather than the late phases in both the frequency and duration of paw-lick ($F_{(6,37)} = 4.827, p < 0.01$; and $F_{(6,37)} = 5.919, p < 0.01$) respectively. Similarly, potentiating effects were observed, nevertheless at both early and late phases in the duration of paw lick ($F_{(6,37)} = 14.016, p < 0.001$; and $F_{(6,37)} = 6.044, p < 0.001$) respectively when 1 mg/kg ketamine was co-administered with diclofenac 75 mg/kg as shown in Table 4.

Table III: Assessment of the Analgesic Effect of Sub-Psychotomimetic Dose of Ketamine (1 mg/kg) at Early Phase (0-5 Minutes) and Late Phase (15-30 Minutes) Post-Treatment Using Formalin Test (n = 6)

TREATMENT	FREQUENCY OF PAW LICK		DURATION OF PAW LICK (seconds)	
	0 - 5 minutes	15 - 30 minutes	0 - 5 minutes	15 - 30 minutes
SALINE 10 ul/kg	17.17 ± 1.76	9.00 ± 1.21	72.5 ± 6.40	51.83 ± 5.96
KETAMINE 1 mg/kg	23.43 ± 2.99	20.71 ± 3.48*	126.57 ± 11.82*	162.86 ± 11.04*
DICLOFENAC 75 mg/kg	17.43 ± 1.15	8.86 ± 3.33	131.14 ± 7.95	78.71 ± 28.12
MORPHINE 10 mg/kg	13.67 ± 1.23	0.00 ± 0.00	53.00 ± 4.26	0.00 ± 0.00

Data presented as mean ± SEM; *P < 0.05 significantly different from saline group

Table IV: Evaluation of the Effect of Sub-Psychotomimetic Dose of Ketamine (1 mg/kg) on Morphine and Diclofenac-Induced Analgesia at Early Phase (0-5 Minutes) and Late Phase (15-30 Minutes) Post-Treatment Using Formalin Test (n = 6)

TREATMENT	FREQUENCY OF PAW LICK		DURATION OF PAW LICK (seconds)	
	0 - 5 minutes	15 - 30 minutes	0 - 5 minutes	15 - 30 minutes
KETAMINE 1 mg/kg	23.43 ± 2.99	20.71 ± 3.48	126.57 ± 11.82	162.86 ± 11.04
DICLOFENAC 75 mg/kg	17.43 ± 1.15	8.86 ± 3.33	131.14 ± 7.95	78.71 ± 28.12
DICLOFENAC 75 mg/kg + KETAMINE 1 mg/kg	13.50 ± 1.73	0.17 ± 0.17	31.50 ± 2.81**	0.33 ± 0.33**
MORPHINE 10 mg/kg	13.67 ± 1.23	0.00 ± 0.00	53.00 ± 4.26	0.00 ± 0.00
MORPHINE 10 mg/kg + KETAMINE 1 mg/kg	4.33 ± 1.96*	0.00 ± 0.00	9.33 ± 4.87*	0.00 ± 0.00

Data presented as mean ± SEM; *p < 0.05 significantly different from morphine group, **p < 0.05 significantly different from diclofenac group

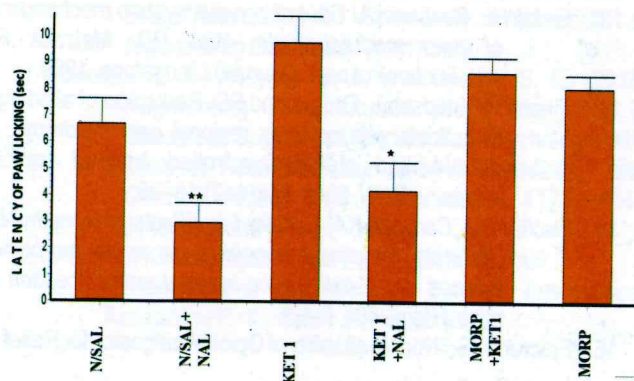


Figure 1 Effect of pre-treatment with naloxone in ketamine-induced analgesia at 90 minutes post-treatment using hotplate test. The columns and vertical bars represent Mean ± SEM respectively (n = 6), *p < 0.05 significantly different from KET 1 group, **p < 0.05 significantly different from N/SAL group

Legend N/SAL = Normal saline 10 ul/g, KET 1 = ketamine 1 mg/kg, MORP = Morphine 10 mg/kg, N/SAL + NAL = N/Saline 10 ul/g + naloxone 1 mg/kg, KET 1 + NAL = ketamine 1 mg/kg + naloxone 1 mg/kg, MORP + KET 1 = Morphine 10 mg/kg + ketamine 1 mg/kg

DISCUSSION

This study assessed possible analgesic effect of a sub-psychotomimetic dose of Ketamine (SPDK), its effects on morphine-induced and diclofenac-induced analgesia using the hot plate and formalin-induced pain models. It further explored the neuronal mechanism of 1 mg/kg ketamine in analgesia in mice. Assessment of the central analgesic effect of the sub-psychotomimetic dose of ketamine was carried out in the hot plate model. The hot plate test is based on induction of pain by thermal stimulus and an increase in the reaction time is generally considered to be an important parameter for evaluating central antinociceptive activity (Rujjanawate *et al.*, 2003) of compounds. The reaction time is measured by parameters such as paw-lick, withdrawal, and jumping off the plate (Le Bars *et al.*, 2001; Almeida *et al.*, 2008), which are considered to be supraspinally integrated responses (Yaksh and Rudy, 1976). The hot plate test is able to differentiate between central opioid-like analgesics and peripheral analgesics (Le Bars *et al.*, 2001). Thus, it is a specific central antinociceptive test (Parkhouse and Pleuvry, 1979).

The sub-psychotomimetic dose of Ketamine (SPDK) (1 mg/kg) significantly increased latency of paw-lick in mice at 90 minutes post-treatment but hitherto was reduced at 60 minutes post-treatment when compared to saline in the hot plate model. This suggested that 1 mg/kg ketamine induced analgesia at 90 minutes post-administration. This result supported the conclusion of several studies that ketamine could induce analgesia (Schmidt *et al.*, 1999; De Kock and Lavand'homme, 2007; López, 2007; Qutaiba *et al.*, 2013 and Owolabi *et al.*, 2018). Although it is expected that ketamine may induce analgesia if at all at relatively shorter time post-administration since it is a short-acting molecule, its pharmacokinetics in analgesia may however be different (Clement and Nimmo, 1981). Albeit, the plausible reason mentioned herein is beyond the scope of this study thus, further research is required. This study also reported that SPDK significantly augmented morphine-induced and diclofenac-induced analgesia at 5 and 60 minutes post-administration when co-administered with morphine and diclofenac respectively. This outcome is similar to the reports of Winters *et al.*, 1988 and Hance *et al.*, 1989 where they claimed that the combination of small-doses of ketamine with morphine showed increased analgesia but however induced catalepsy. Meanwhile, Edward *et al.*, 1993; Javery *et al.*, 1996 and Suzuki *et al.*, 1999 concluded that iv administration of low dose(s) ketamine with morphine increased analgesia and less adverse effects thus, useful in post-operative pain management. Similar results were also obtained when low-dose ketamine was used as an adjunct to local anesthetics, opioids (Qutaiba *et al.*, 2013) or other analgesic agents, such as meperidine (Parkhouse and Pleuvry, 1979) or methadone (Fischer *et al.*, 2005).

Further, the neuronal mechanism of analgesia of SPDK was determined by pre-treating SPDK with naloxone, a μ [μ] opioid

receptor antagonist (Tjolsen *et al.*, 1992) in the hot plate model. The ketamine-induced analgesia was significantly reversed by 1 mg/kg naloxone, thereby, implicating the μ opioid receptor in modulating the effect of the former. This suggested the involvement of opioid receptors in ketamine-induced analgesia thus corroborated the outcome of studies demonstrated by Pekoe and Smith, 1982. Also, Howe *et al.*, 1983 suggested that ketamine may produce antinociception through interaction with spinal μ receptor, NMDA receptor antagonism, and activation of the descending pain inhibitory monoaminergic pathways, which is expressed by alpha2-adrenoceptors at the spinal level. However, it is likely that they mimic the endogenous endorphin/enkephalin system, one function of which is likely to be an intrinsic mechanism of pain control (Fields and Basbaum, 1999; and Julius and Basbaum, 2001).

More so, the analgesic effect of ketamine was assessed using the formalin-induced paw-lick model (Vogel, 2002). Formalin test is a valuable tool for assessing the analgesic properties of drug candidates (Le Bars *et al.*, 2001; and Sawynok and Liu, 2003). It has been proposed as a chronic pain model in rats which is sensitive to centrally active analgesic agents (Dubuisson and Dennis, 1977). It is believed to resemble clinical pain more closely in comparison with other tests that employ mechanical or thermal stimuli (Tjolsen *et al.*, 1992, Almeida *et al.*, 2008). It is considered a model of nociceptive response in two distinct phases involving different mechanisms, viz: neurogenic pain and inflammatory pain phases (Murray *et al.*, 1988 and Rujjanawate *et al.*, 2003). The first phase (neurogenic pain) results from the direct chemical stimulation of myelinated and unmyelinated nociceptive afferent fibres, mainly C fibres, and the release of substance P (Le Bars *et al.*, 2001), which can be suppressed by opioid analgesic drugs like morphine.

Contrarily, SPDK did not elicit analgesic effect at all the phases of the formalin-induced pain model rather, allodynic effect was observed. Although topical ketamine is known to alleviate allodynic pain (Finchet *et al.*, 2009 and Carlton, 2009), its effect herein clearly contradicted this claim. This is however not unexpected, since NMDA receptors exist primarily in the CNS; and pharmacologically, ketamine is a centrally-acting agent, it is therefore least expected to mitigate peripheral nociception. Nevertheless, ketamine significantly augmented both morphine- and diclofenac-induced analgesia in this model similar to the result obtained in the hot plate model. Perhaps, this role of ketamine in analgesia may be attributed to its ability to bind to opioid receptors as suggested by Smith *et al.*, 1980; Howe *et al.*, 1983; and Pekoe and Smith, 1982.

CONCLUSION

This study showed that sub-psychotomimetic dose of ketamine possessed analgesic effect, and potentiated both morphine-induced and diclofenac-induced analgesia possibly mediated through modulation of opioidergic pathway in mice. However, further studies on the safety profile of acute and chronic administrations of SPDK; and receptor characterization of antinociceptive effect of ketamine are thus suggested.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgment

The authors acknowledged the Management of University of Ilorin,

Ilorin, Nigeria for the Award of Staff Development Grant to support this study. Also, we acknowledged the staff of the Department of Pharmacology, OAU, Ile-Ife, for the care of the animals used in this study.

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