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Research Article

Standardisation and Validation of Acute and Chronic Administration Ketamine at Different Doses to Produce Psychosis Like Behavioural Changes in Mice

Monu Yadav[1], Milind Parle[1], Mamta Sachdeva[2], Sameer Dhingra[3*]

[1]*Faculty of Medical Sciences, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar-125001, India*
[2]*University Institute of Pharmaceutical Sciences, UGC Center of Advanced Study (UGC-CAS) in Pharmaceutical Sciences, Panjab University, Chandigarh, India*
[3]*Faculty of Medical Sciences, School of Pharmacy, The University of the West Indies, St. Augustine, Trinidad and Tobago*

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ABSTRACT
Psychosis is a complex mental illness, characterised by positive, negative and cognitive symptoms. NMDA receptor antagonists have been established to induce behavioural as well as biochemical changes in rodents similar to psychotic patients. The aim of the present study was to investigate the effective dose and treatment period of ketamine to induce some behavioural changes. The results suggest that acute treatment of ketamine (50 and 100 mg/kg, i.p.) induced hyperlocomotor activity and reduced step down latency time in passive avoidance test, whereas in effective in forced swim test. Further, with the chronic administration of ketamine (50 and 100 mg/kg, i.p.) effective to produced hyperlocomotor activity, reduced the step down latency time in passive avoidance test and enhanced the immobility duration in forced swim test. Moreover, these behavioural changes persisted for 7 days after the treatment period. Thus, our findings suggest that the chronic administration of Ketamine (50 and 100 mg/kg, i.p.) potential to produce behavioural changes, would serve as an effective tool to screen antipsychotic drugs.

Keywords: Ketamine, NMDA receptor, psychosis, animal models.

INTRODUCTION

Psychosis is a complex, debilitating mental disorder that affects large number of population[1]. Usually, psychosis is categorized into three main symptoms, positive, negative and cognitive symptoms[2,3]. Bizzare behaviour, delusions, hallucinations and loss of contact from reality collectively come under positive or bizarre symptom of psychosis[4,5]. Alogia, anhedonia, avolition, flat affect or social withdrawal or loss of emotion are negative symptom of psychosis[6,7]. Cognitive symptoms represents as loss of concentration and lack of learning or memory process[8]. Animal models are used to under the exact mechanism of action as well as for screening of new test drugs[9,10,11]. The limited research on psychosis has been the main reason of lack of appropriate animal model that mimics the clinical symptoms of psychosis[12]. Several chemicals or drugs, used to induce psychosis in rodents are not capable to imitate the symptoms seen in human. N-methyl d-aspartate (NMDA) receptor antagonists such as dizocilpine, Ketamine and phencyclidine are used to produce psychosis in rodents as well as in human[13]. Ketamine, an arylcyclohexylamine derivative of phencyclidine is non-competitive NMDA receptor antagonist and stimulates psychotic symptoms[14]. It has also been observed that Ketamine induce behavioural and biochemical alterations in rodents[15,16]. Though, the appropriate dose for inducing psychosis in rodent is still not well defined. In literature, various doses are used to induce psychosis however these doses are not standardised and validated. Therefore, this study was carried out to standardise and validate acute and chronic administration of different doses of Ketamine to induce psychotic symptoms in mice.

MATERIALS AND METHODS

Animal protocol

Swiss albino mice of either sex, weighing 20-25g were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India. The experimental protocol was approved by Institutional Animals Ethics Committee and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India (Registration no. 0436).

Drug protocol

Ketamine was obtained from Neon Pharmaceutical Pvt. Ltd, India and diluted with9%w/v saline for use.

Experimental design

Animals were divided into 4 groups with 6 mice in each group:

Group 1: Control animals treated with vehicle only.

**Author for Correspondence: sameerdhingra78@gmail.com*

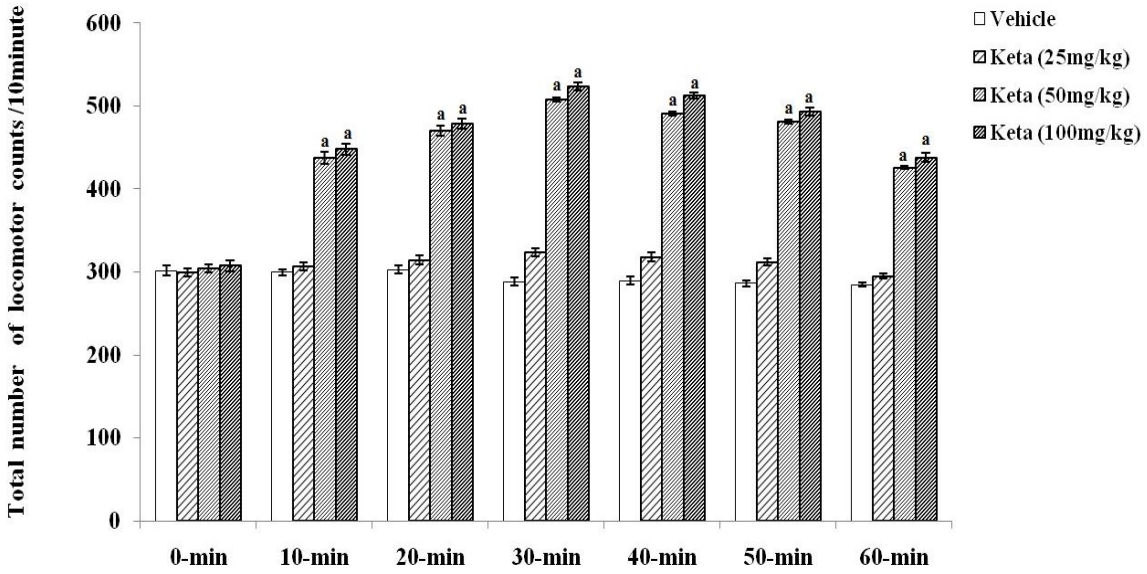

Figure 1: Effect of ketamine on locomotor activity.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes $p<0.001$ as compared to vehicle treated group.

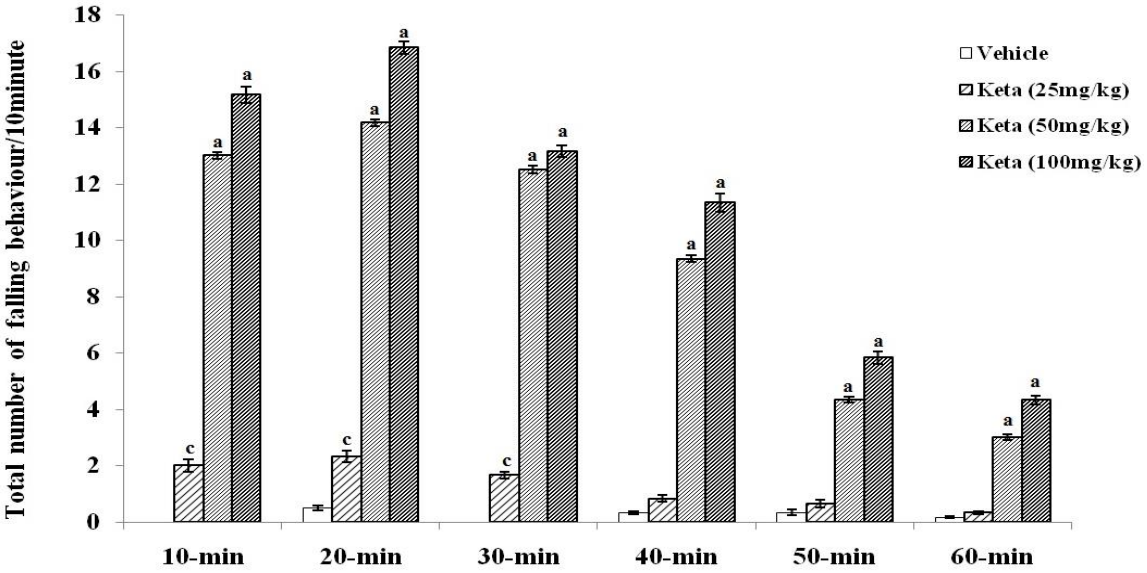

Figure 2: Effect of ketamine on falling behaviour.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes $p<0.001$ and 'c' denotes $p<0.05$ as compared to vehicle treated group.

Group 2, 3 and 4: Animals treated with Ketamine 25, 50 and 100 mg/kg, i.p.

Behavioural tests

In the present study, Ketamine (25, 50 and 100 mg/kg, i.p.) were used to induced stereotypic behaviours, positive, negative and cognitive symptom of psychosis in mice. After acute and chronic administration of ketamine, behavioural tests were performed.

Locomotor activity

Locomotor activity was counted using actophotometer (INCO, Ambala, India). Animal was placed for 5 minute in activity chamber before assessing actual locomotor activity. Total locomotor counts were count per 10 minute over 120 minutes for 5 minutes[17].

Stereotypic behaviour

Ketamine treatment produced stereotypic behaviours (falling, turning, head bobbing and weaving) in animals. Mouse was placed into a plastic cage (37 × 24 × 30 cm)divided into quadrants by lines on the floor of the cage and allowed for acclimatization for 30 minute before began the test. Total number of falls (falling),turn around (turning), neck wave left, right, up and down (head bobbing) and grooming and rearing behaviours (weaving) were counted every 10 minutes over 60 minute[18].

Forced swim test

In animals, increased immobility period is a sign of negative or depressive symptoms. Mice were individually into FST chamber (25×12× 25 cm) filled with water (23±2℃) up to 15 cm and forced to swim. After 2 minute of vigorous swimming, mouse showed immobility with

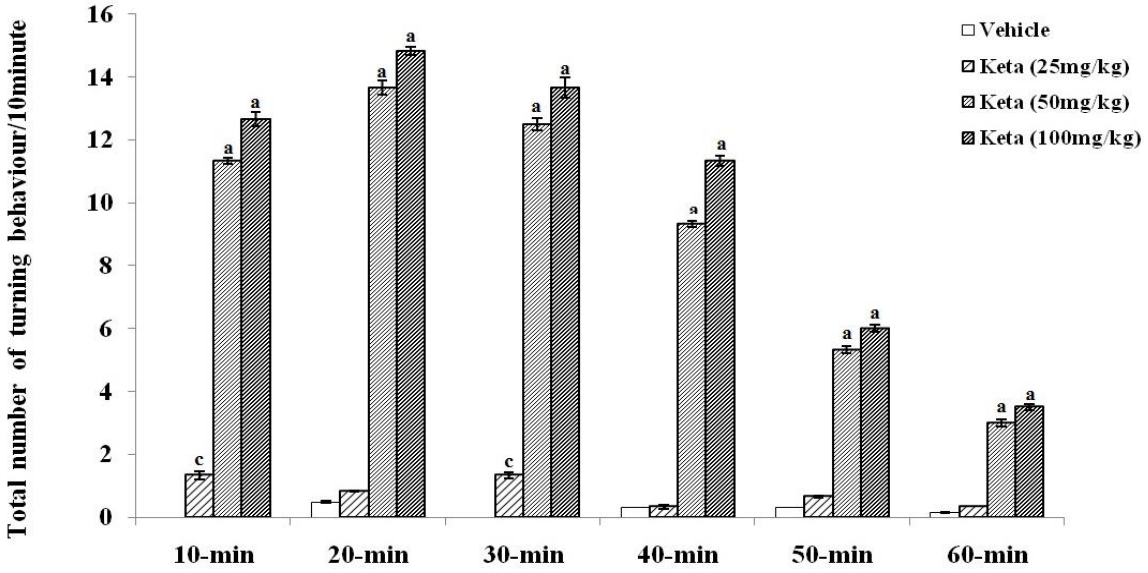

Figure 3: Effect of ketamine on turning behaviour.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

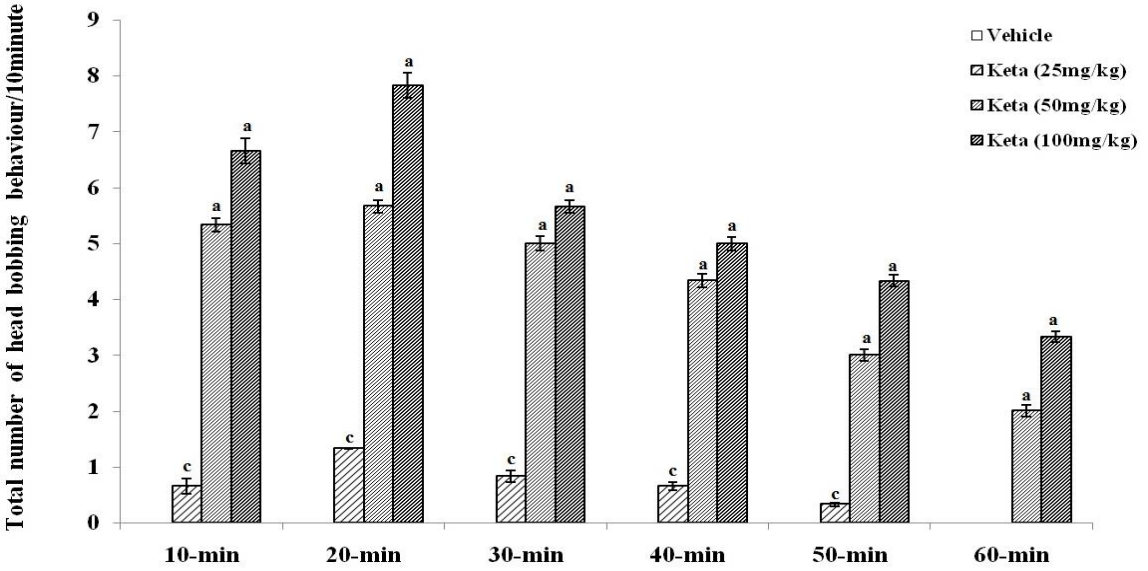

Figure 4: Effect of ketamine on head bobbing behaviour.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

minimum movements. Total time of immobility in next 4 minute of test was recorded[16, 19].

Passive avoidance test

Step down latency on passive avoidance test has been used to assess long term memory. The apparatus consists of an electric grid with a platform (shock-free zone) in the centre. The eexperiment was performed in learning and memory sessions. The electric shock was given and latency to reach the shock-free zoneis recorded during learning and memory sessions. The time taken by the animal to step down on floor with all its paws is known as Step Down Latency (SDL). After 24 hour of learning or training sessions, the memory or testing session was performed; shocks were not delivered and SDL was recorded in 5 minute[20].

Statistical analysis

Results were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance followed by Tukey's tests using Graph Pad Instat, p< 0.05 was considered as significant.

RESULTS

Effect of ketamine on locomotor activity in actophotometer

This study was carried out to investigate the effect of Ketamine indifferent dose (25, 50 and 100mg/kg, i.p) for producing hyperlocomotor activity. Administration of Ketamine (50 and 100mg/kg i.p) for 7 successive days was effective to produced hyperlocomotor activity in mice as compared to vehicle treated group (Figure 1).

Effect of ketamine on stereotypic behavior in mice

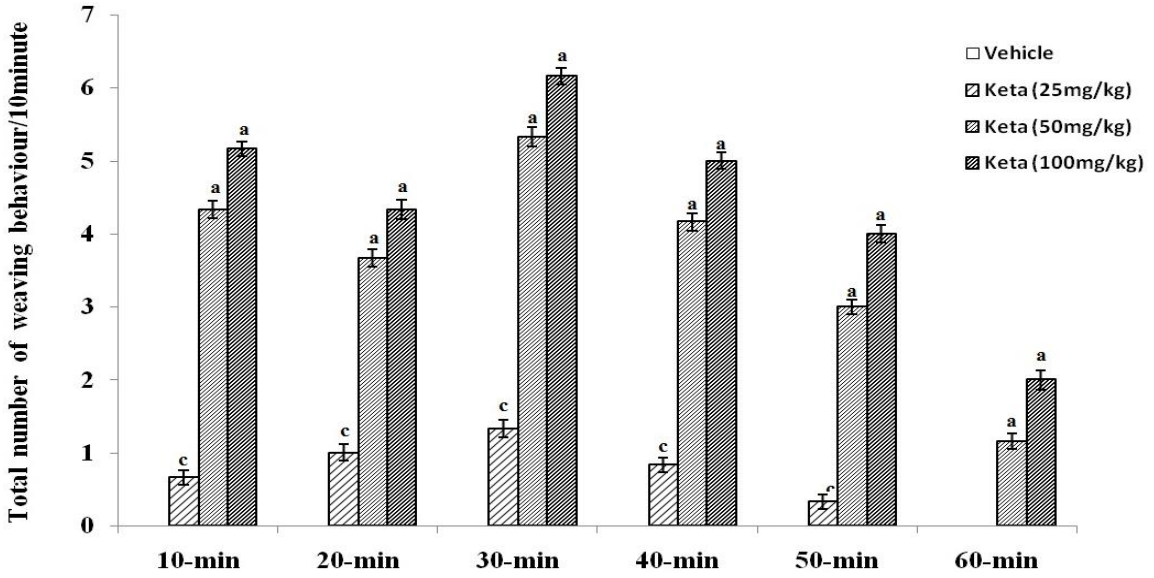

Figure 5: Effect of ketamine on weaving behaviour.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

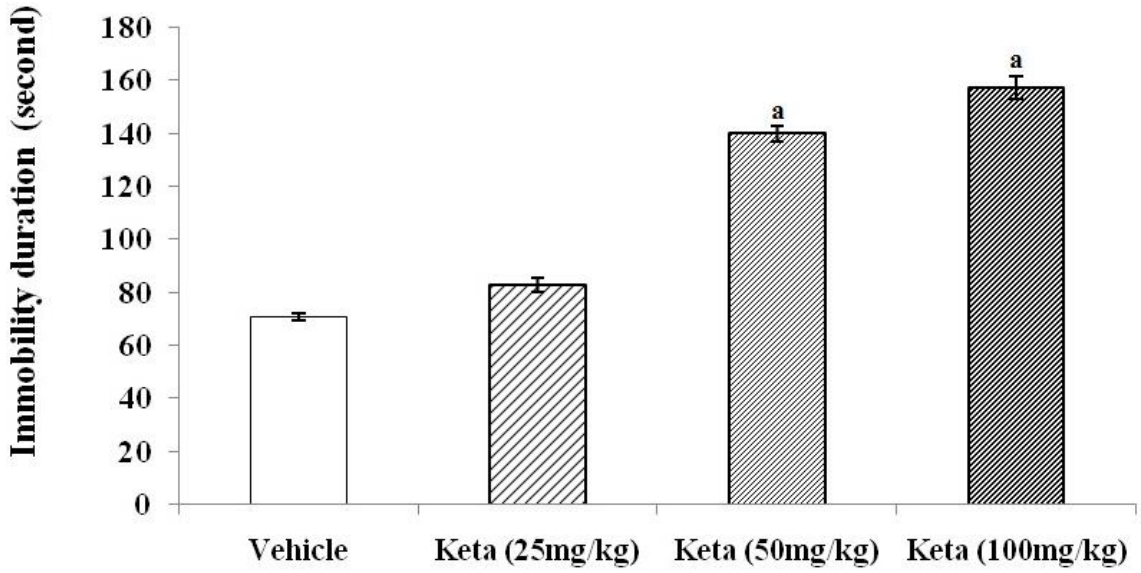

Figure 6: Effect of ketamine on immobility period.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

Ketamine (25, 50 and 100 mg/kg, i.p.) was administered to mice for 10 days, on the 10th day they were exposed for stereotypic behaviours (falling, turning, head bobbing and weaving). Ketamine (50 and 100 mg/kg, i.p.) were significant (p<0.001) to induce stereotypic behaviours as compared to vehicle treated group (Figure 2, 3, 4 and 5).

Effect of ketamine on immobility period in forced swim test (FST)

Immobility in FST shows anhedonia, a negative symptom of psychosis. Ketamine (25, 50 and 100 mg/kg, i.p.) was administered to mice for 12 days, on the 12th day FST was performed. Ketamine (50 and 100 mg/kg, i.p.) significantly (p<0.001) to increased immobility period as compared to vehicle treated group (Figure 6).

Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test. Keta=Ketamine 'a' denotes p<0.001 as compared to vehicle treated group.

Effect of of ketamine on step down latency (SDL) in passive avoidance test

Ketamine (25, 50 and 100 mg/kg, i.p.) was administered for 14 days, on the 14thday passive avoidance test was performed to investigate its effect on memory. Ketamine (50 and 100 mg/kg, i.p.) were significant (p<0.001) todecrease SDLas compared to vehicle treated group (Figure 7).

Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.

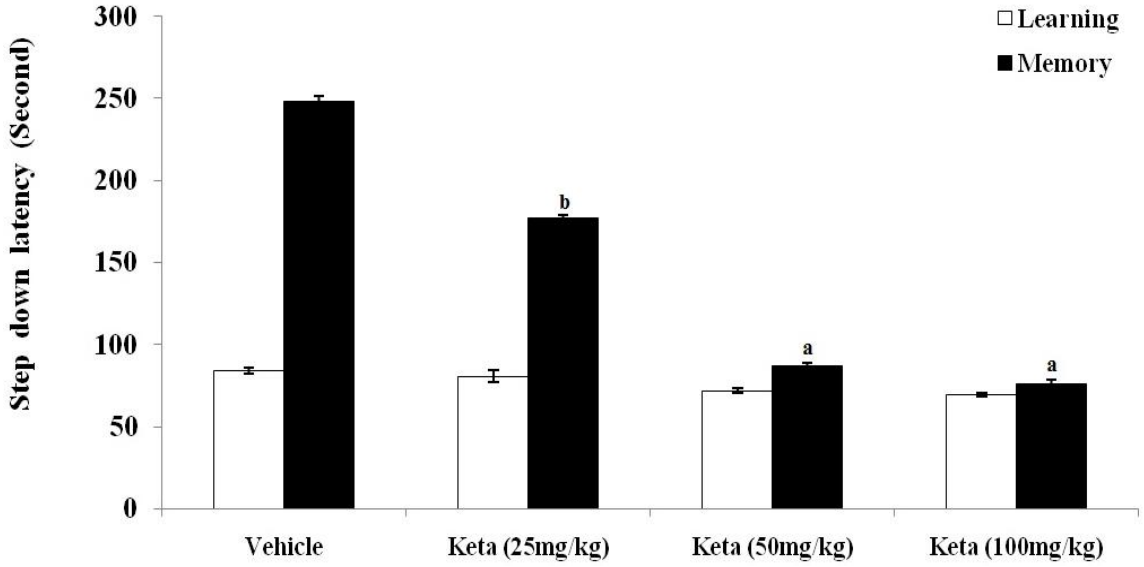

Figure 7: Effect of ketamine on step down latency.
Values are expressed as mean ± SEM (n=6). Results were analyzed by one-way ANOVA followed by Tukey's test.
Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

Keta=Ketamine 'a' denotes p<0.001 and 'c' denotes p<0.05 as compared to vehicle treated group.

DISCUSSION

Psychosis is a chronic mental illness characterized by several behavioural changes[1,18]. The present study was designed to standardized and validate Ketamine dose to induce stereotypic behaviours, positive, negative and cognitive symptom of psychosis in mice. In the present study, it has been observed that acute administration of ketamine at different dose (25, 50, 100 mg/kg, i.p.) showed dose depended hyperlocomotor activity and stereotypic behaviours. It has been reported that NMDA receptors antagonist inhibit NMDA receptors located on GABAergic neurons leading to exaggerate the dopamine level in the limbic and subcortical regions of brain and believed to increase the locomotor activity and stereotypic behaviours[15, 16,18]. The increased immobility period after chronic treatment with PCP has been used earlier to induce negative symptoms (avolition and flattening affect) of psychosis. In this study, we have investigated that chronic treatment with Ketamine at the dose of 50 and 50 mg/kg, i.p., for 12 days induced dose dependently increased of immobility period in FST. This effect was not found after sub-acute treatment with ketamine. In previous studies, it has been seen that induction of negative symptoms with chronic administration of Ketamine could be attributed due to activation of dopaminergic turnover in cortex and striatum resulting to increased serotonin production and activation of serotonergic system in cortex[21]. Furthermore, in the current study, chronic administration of Ketamine at the dose of 50 and 100mg/kg, i.p., were effective to disturb the memory or cognitive functions as indicated by decreased step down latency in passive avoidance test. In contrast with several reports, it has been found that involvement of dopaminergic system in the cognitive deficits. Moreover, ketamine act on non-NMDA neurotransmitter systems, it suppressed the acetylcholine level in the hippocampus and activates acetylcholinesterase enzyme and induce memory dysfunction[22].There, this study demonstrated that Ketamine at the dose of 50 and 100 mg/kg, i.p., induced behavioural changes in mice that mimics the core symptoms (positive, negative and cognitive symptoms) of clinical psychosis and symptoms persists after the treatment period. As it has observed that typical or 1st generation of drugs are effective in stereotypic behaviours and positive symptoms while, atypical or 2nd generation antipsychotic drugs are effective in stereotypic behaviours, positive, negative and cognitive symptoms. Thus, Ketamine (50 and 100 mg/kg, i.p.) would be effective to screen both category of antipsychotics.

CONCLUSION

The present study proposed that the chronic administration of Ketamine (50 and 100mg/kg, i.p.) has the potential to induced behavioural changes associated with clinical symptoms of psychosis. Therefore, Ketamine administration chronically at the dose of 50 or 100 mg/kg, i.p. would be a useful model to screen new antipsychotic potential compounds.

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