

# Evaluation of protective role of N-methyl-D-aspartate receptor antagonist against Parkinson's model(s) in rat

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## ABSTRACT

**Aim:** The objectives of the present research were to observe the effect of N-methyl-D-aspartate (NMDA) receptor antagonist on Parkinson model(s) in rats and to compare the efficacy of NMDA receptor antagonist with standard antiparkinson drug. **Materials and Methods:** Albino rats were divided into five experimental groups of six each. 24 animals except normal and acute dose of haloperidol (1 mg/kg) were administered intraperitoneally for induction of catalepsy. Six animals received saline 0.9% NaCl and served as normal saline. On the 20<sup>th</sup> day, catalepsy was evaluated by reported instruments. Levodopa (150mg/kg) was used as standard drug and kynurenic acid (50 mg/kg and 100 mg/kg) was used as test drug. Different instrument utilized in the study was catalepsy bar test, actophotometer, and rotarod. **Results and Discussions:** The results showed that acute administration of kynurenic acid (50 mg/kg and 100 mg/kg) significantly reduced catalepsy, grooming and significantly increased motor coordination, locomotor activity. **Conclusion:** It was concluded from the study that kynurenic acid ameliorates the symptoms of Parkinson's disease in rats.

**Key words:** Actophotometer, catalepsy bar test, haloperidol, kynurenic acid, N-methyl-D-aspartate receptor antagonist, Parkinson's disease, rotarod

## INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder affecting patients in large numbers throughout the world.<sup>[1]</sup> The first description of PD was given by James Parkinson in early 19<sup>th</sup> century.<sup>[2]</sup> An estimated 10 million people in the world (i.e. approximately 0.3% of the world population) and 1% of those above 60 years are found to be affected with PD.<sup>[3]</sup> PD is the second most common neurodegenerative disorder of aging and the most common movement disorder. Characterized clinically by resting tremor, bradykinesia, rigidity, and postural instability.<sup>[4]</sup> The pathological hallmark of the illness is a relatively selective degeneration of the neuromelanin-pigmented dopaminergic neurons of the substantia nigra pars compacta coupled with the formation of intracytoplasmic protein aggregates known as Lewy bodies.<sup>[5,6]</sup> Progressive loss of dopaminergic neurons is a feature of normal aging; however, symptoms of PD coincide with excessive loss (70–80%) of these neurons.<sup>[7]</sup> Without treatment, PD

progresses over 5–10 years to a rigid, akinetic state in which patients are incapable of caring for themselves.<sup>[8,9]</sup> Speech impairment has been reported in 60–80% of the PD patients which reaches up to 100% in the later stages.<sup>[10]</sup> Memory disturbances and dementia are known to occur in later stages of PD. Patients with early PD can have subtle disturbances in neuropsychological testing.<sup>[11]</sup> The prevalence of depression in PD patients ranges from 7.7 to 76%.<sup>[12,13]</sup>

The patient suffering from PD constitute a heavy burden on the society as well as healthcare system.<sup>[14]</sup> Therefore, there is an ever-increasing need for effective pharmacotherapy and recent research efforts have come up with novel therapeutic agents in the treatment of neurodegenerative diseases. Among

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these, blockers of glutamate release or of glutamate receptors specifically N-methyl-D-aspartate (NMDA) receptors, have shown considerable importance as potential neuroprotective agents.<sup>[15]</sup>

Kynurenic acid is neuroactive and modulates glutamate, dopamine, and acetylcholine neurotransmission. It is known to be anticonvulsive and protect against neurotoxicity, as well as influence dopamine activity, cognition and sensorimotor gating functions in animals.

The objectives of present research work were to observe the effect of NMDA receptor antagonist on Parkinson model(s) in rats and to compare the efficacy of NMDA receptor antagonist with standard antiparkinson drug.

## MATERIALS AND METHODS

Kynurenic acid was obtained from Chempure Wilson Laboratons Chemicals Unlimited, Mumbai. Haloperidol and levodopa were procured from Taj Pharmaceuticals, Mumbai.

### Procurement of Experimental Animals

The albino rats weighing 180–200 g, bred in the animal house of Institute of Pharmaceutical Education and Research (IPER), Wardha, were procured. The animals were housed in polypropylene cages at a temperature of  $22 \pm 2^\circ\text{C}$  with relative humidity of 40–60% for 12 h in light-dark cycle. Animals were fed with a balanced diet and water *ad libitum* during the complete experimental period. The experimental design and animal handling and disposal procedure were approved by the institutional animal ethical committee of IPER, Wardha (535/02/a/CPCSEA/Jan2002).

### Induction of Catalepsy

Acute dose of haloperidol (1 mg/kg) was administered intraperitoneally for the induction of catalepsy for 20 days.

### Catalepsy Bar Test

Bar test determinations were carried out by gently placing rat's forepaw over a horizontal bar, fixed at the height of

10 cm with heads of animals toward upward on an inclined surface at an angle of  $60^\circ$  with the hind limbs abducted. A horizontal glass bar having (2 mm diameter) elevated 4.5 cm above the observation floor. The length of time during which the animal retained this position was recorded by measuring the time from the placement of the rat until removal of one of its forepaws. Testing was performed 60 min postinjection and the time to withdrawal of legs by the rats was measured.<sup>[16]</sup>

### Actophotometer

The locomotor activity can be easily measured using actophotometer. It operates on photoelectric cells that are connected in a circuit with a counter. When a beam of light falling on photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square area in which animal moves. Effect on locomotor activity was measured every 60 min for 10 min thereafter up to a total duration of 3 h.<sup>[17,18]</sup>

### Rotarod

The rotarod apparatus was used in two different acceleration modes, gradually increasing either from 4 to 30 rpm (slow speed) or from 5 to 35 rpm (fast speed) over the course of 5 min. Rats were placed on the apparatus and rotation was initiated. Latency to fall was recorded automatically.<sup>[19,20]</sup>

### Grooming

Rats were housed individually for 24 h before the experiment. Trials were given within the last 4 h of the light phase of the light/dark cycle. Rats were injected intraperitoneally; food and water were removed from the cage. Each rat was observed once per minute and its activity was recorded (activities included locomotion, grooming, nibbling on bedding or feces, digging in bedding, and stationary/sleeping). The proportion of each type of activity observed from 15 to 115 min after injection was determined and analyzed.

### Experimental Protocol

All animals were divided into five experimental groups of six each. 24 animals except normal were administered haloperidol for the induction of catalepsy. Six animals received saline 0.9% NaCl and served as normal saline. Catalepsy was evaluated by reported instruments on the 20<sup>th</sup> day [Table 1].

### Statistical Analysis

Data were analyzed using Prism 5 for Windows (version 5.03). The result was expressed as mean  $\pm$  standard error of the mean. One-way analysis of variance, Tukey's multiple

**Table 1: Animal groups**

Groups	Treatments
Group I	Normal (saline 0.9% NaCl)
Group II	Control (Haloperidol 1 mg/kg, i.p) <sup>[21]</sup>
Group III	Kynurenic acid (50 mg/kg, i.p)
Group IV	Kynurenic acid (100 mg/kg, i.p) <sup>[22]</sup>
Group V	Levodopa (150 mg/kg, i.p) <sup>[21]</sup>

comparison test were used to test the significance of the difference between the variables in various groups.  $P < 0.05$  was considered to be statistically significant.

## RESULTS AND DISCUSSIONS

### Catalepsy Bar Test

Table 2 and Figures 1-3 depict the effect of different doses of kynurenic acid on haloperidol-induced catalepsy. Treatment with kynurenic acid and levodopa decreased the haloperidol-induced catalepsy compared to control. Although the effect showed by kynurenic acid was less as compared to standard drug levodopa, still results of kynurenic acid were encouraging.

### Actophotometer

Table 3 and Figures 4-6 show the effect of different doses of kynurenic acid on haloperidol inhibited locomotor activity.

Treatment with kynurenic acid and levodopa increased the haloperidol inhibited locomotor activity as compared to control. Effect of kynurenic acid was less than standard drug levodopa, but antiparkinson's effect of kynurenic acid was comparable to levodopa.

### Rotarod

Table 4 and Figures 7-9 depict the effect of different doses of kynurenic acid on haloperidol inhibited locomotor activity. It was seen that kynurenic acid and levodopa reversed the haloperidol inhibited locomotor activity as compared to control. Effect received with standard drug levodopa was good than kynurenic acid, but antiparkinson's effect of kynurenic acid was comparable to levodopa.

### Grooming

Table 5 and Figure 10 illustrate the effect of different doses of kynurenic acid on haloperidol-induced grooming. It

**Table 2: Effects of kynurenic acid on catalepsy (after 60 min)**

Groups	Treatments	After 60 min catalepsy time (s)	After 120 min catalepsy time (s)	After 180 min catalepsy time (s)
Group I	Normal	05.2±1.4	08±1.4	07±0.9
Group II	Control	24.0±1.7	29.0±2.4	27.8±1.7
Group III	Kynurenic acid	21.7±1.7*	19.2±1.3*	18.0±2.4*
Group IV	Kynurenic acid	20.0±1.5**	14.1±1.5**	11.0±1.3**
Group V	Levodopa	11.0±1.6***	09.7±1.8***	08±1.4***

Values expressed as mean±SEM,  $n=6$ , one-way followed by Tukey's multiple comparison test, \* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$ , compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance

**Table 3: Effect of kynurenic acid on locomotor activity**

Groups	Treatments	After 60 min number of locomotor count	After 120 min number of locomotor count	After 180 min number of locomotor count
Group I	Normal	297.0±14.3	319.0±26.2	360.0±23.5
Group II	Control	224.0±18.9	154.0±17.4	145.8±13.1
Group III	Kynurenic acid	180.0±33.8*	196.0±23.5*	199.2±26.5*
Group IV	Kynurenic acid	203.0±16.3**	215.0±17.7**	250.0±20.9**
Group V	Levodopa	274.2±16.9***	285.0±21.3***	294.0±22.2***

Values expressed as mean±SEM,  $n=6$ , one-way ANOVA followed by Tukey's multiple comparison test, \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ , compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance

**Table 4: Effect of kynurenic acid on motor coordination**

Groups	Treatments	After 60 min time spent on rotarod (s)	After 120 min time spent on rotarod	After 180 min time spent on rotarod
Group I	Normal	245.8±29.9	255.0±30.1	269.2±24.3
Group II	Control	69.0±14.1	60.3±13.4	54.0±14.1
Group III	Kynurenic acid	86.0±12.4*	97.0±16.1*	115.0±17.9*
Group IV	Kynurenic acid	101.2±19.5**	125.4±15.8**	146.0±17.5**
Group V	Levodopa	170.0±17.4***	179.9±17.5***	199±18.1***

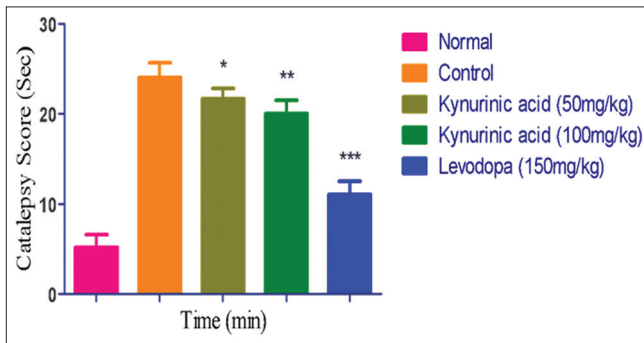
Values expressed as mean±SEM,  $n=6$ , one-way ANOVA followed by Tukey's multiple comparison test, \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ , compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance

was found that kynurenic acid and levodopa decreased the haloperidol-induced grooming compared to control.

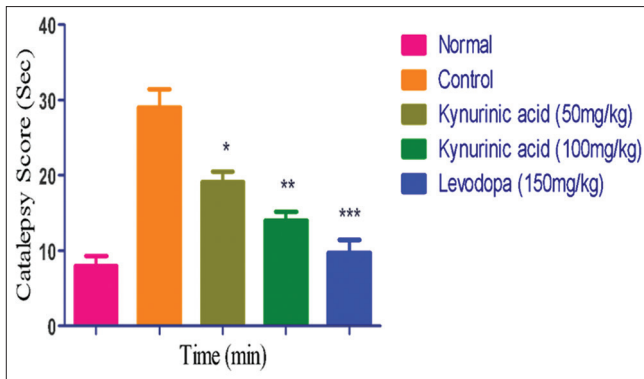
**Table 5: Effects of kynurenic acid on grooming**

Groups	Treatments	Grooming time
Group I	Control	267.8±10.41
Group II	Kynurenic acid	221.3±11.82
Group III	Kynurenic acid	191.7±15.15
Group IV	Levodopa	103.7±12.41

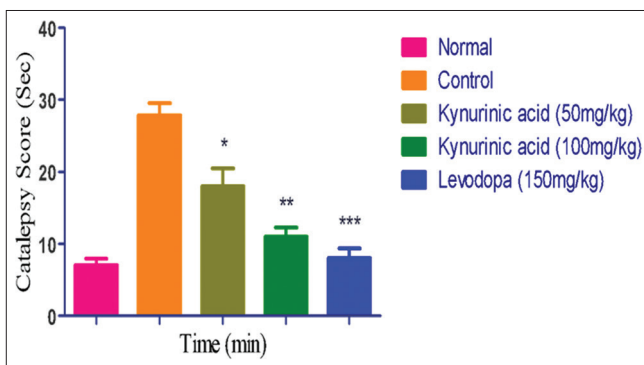
Values expressed as mean±SEM, n=6, one-way ANOVA followed by Tukey's multiple comparison test, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance



**Figure 1: Effects of kynurenic acid on catalepsy (after 60 min)**



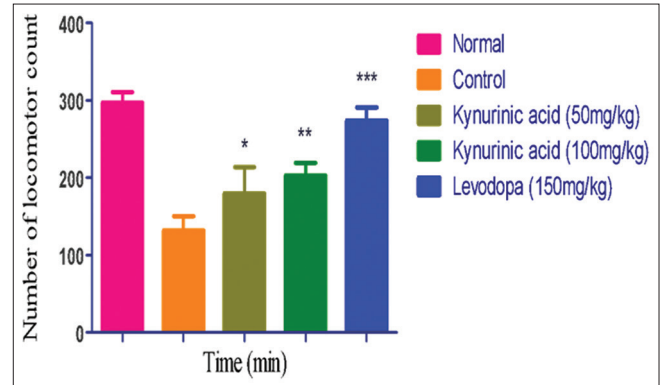
**Figure 2: Effects of kynurenic acid on catalepsy (after 120 min)**



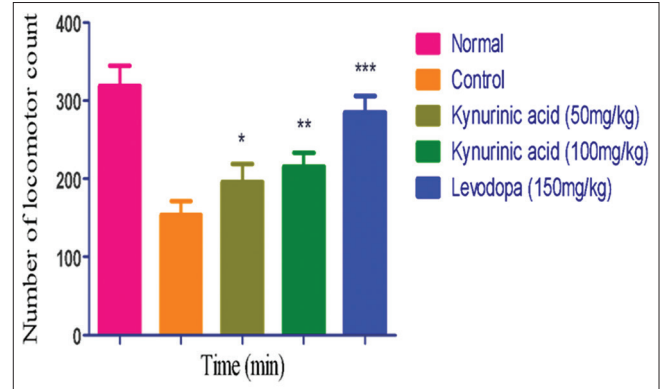
**Figure 3: Effects of kynurenic acid on catalepsy (after 180 min)**

In the present study, the effects of kynurenic acid on extrapyramidal symptoms such as catalepsy, motor coordination, and grooming were the key parameters found in PD were studied. The results revealed that kynurenic acid at doses of 50 mg/kg and 100 mg/kg exhibited the anticataleptic activity in haloperidol-induced catalepsy, reversed the haloperidol inhibited locomotor activity and motor coordination, reduced the haloperidol-induced grooming.

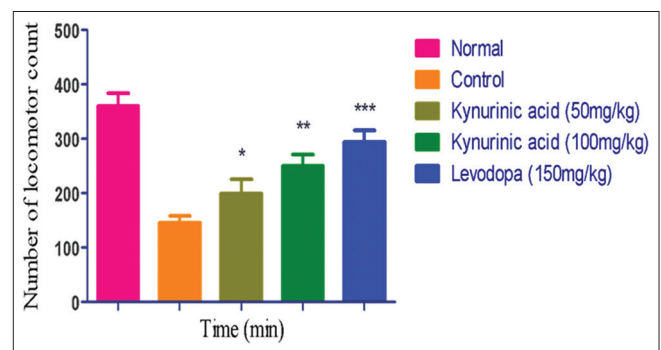
It was also found that the standard drug levodopa and the test drug kynurenic acid at 50 mg/kg and 100 mg/kg dose showed



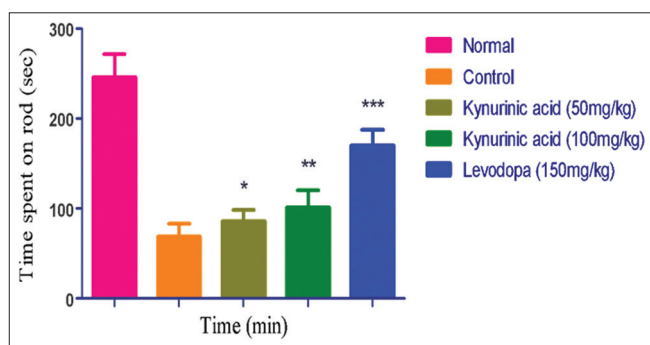
**Figure 4: Effect of kynurenic acid on locomotor activity (after 60 min)**



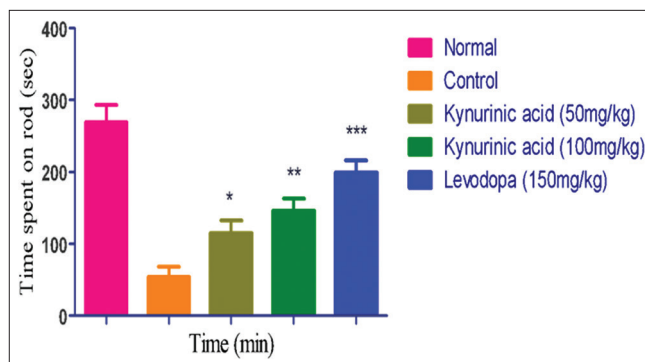
**Figure 5: Effect of kynurenic acid on locomotor activity (after 120 min)**



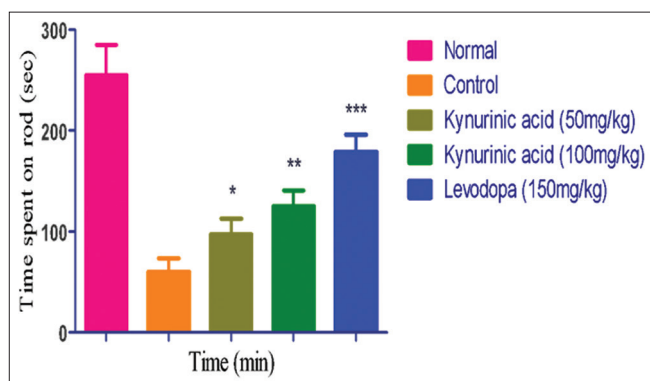
**Figure 6: Effect of kynurenic acid on locomotor activity (after 180 min)**



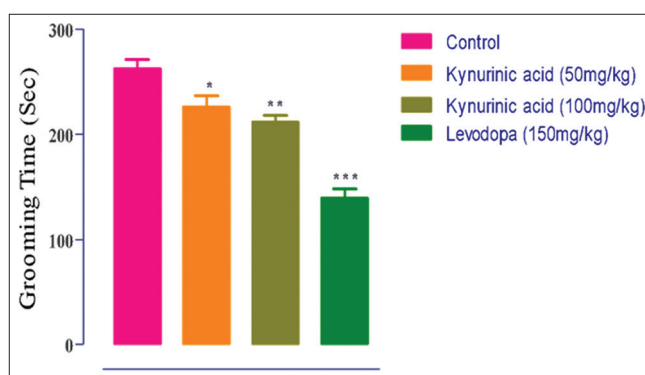
**Figure 7:** Effect of kynurenic acid on motor coordination (after 60 min)



**Figure 9:** Effect of kynurenic acid on motor coordination (after 180 min)



**Figure 8:** Effect of kynurenic acid on motor coordination (after 120 min)



**Figure 10:** Effects of kynurenic acid on grooming

a reduction in the cataleptic scores when observed at the end of 60, 120, and 180 min after the last dose of haloperidol administration. In the subsequent observations, it was found that as the dose of kynurenic acid increased reductions in the cataleptic scores at all the doses used. Moreover, the reduction in cataleptic score was dose and time dependent and comparable to the reduction shown by the standard drug levodopa.

## CONCLUSION

From the study, it was concluded that kynurenic acid ameliorates the symptoms of PD in rats. The mechanism by which the amelioration takes place may be attributed to one or more pharmacological mechanisms, namely, kynurenic acid acts as neuroactive compound and preventing the formation of neurotoxic compounds may have potential therapeutic significance in PD and may enhance the bioavailability of circulatory dopamine by upregulation of dopaminergic signaling.

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