Research Article

Behavioural Assessment of Nicotinic Acid, A GPR109a Receptor Agonist against Memory Deficit in Rats

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ABSTRACT

Present study was aimed at evaluating the neuroprotective role of nicotinic acid, GPR109a receptor agonist against aluminium chloride-induced dementia in rats. Dementia was induced in rats by chronic administration of aluminium chloride (10mg/kg, i.p.) for 42 days. Rats developed dementia as manifested by increased escape latency, total distance travelled to reach the target quadrant, latency to target quadrant in Morris water maze test. Fifteen days chronic oral treatment with nicotinic acid was able to reverse the elevated escape latency at higher doses (100 and 200 mg/kg), but found statistically insignificant. At 50 mg/kg, nicotinic acid failed to show any promising effect on behavioural parameters. Further, at 200 mg/kg dose, nicotinic acid reversed the elevated total distance and latency to target quadrant. However, statistically it was found insignificant as compared to $AlCl_3$ control. This suggest the possibilities of nicotinic acid, a GPR109a receptor agonist, to have neuroprotective action against aluminium-induced memory deficit. However, detailed investigation is required to substantiate the above findings.

Keywords: Nicotinic acid, GPR109a receptor, Aluminium chloride, Dementia, Morris water-maze.

INTRODUCTION

Brain regulates most of the body's functions by neuronal signalling. Any disturbance in the brain due to reasons such as nutritional deficiency, environmental toxins, genetic mutations and formation of toxic molecules (free radicals) may lead to neuronal degeneration.[1,2] Neurodegeneration and resulted depletion of neuronal activity in one or discrete parts of the brain has been is the pathological cause behind various brain disorders. This neurodegeneration presents as motor symptoms (manifesting as bradykinesia, tremor and rigidity), non-motor symptoms originated in cortical (psychosis and memory impairment), basal ganglia (abnormal impulse control, apathy and restlessness), brainstem (anxiety, depression and insomnia), and peripheral nervous system (constipation, abdominal pain, orthostatic hypotension and sense disturbances).[1]

Alzheimer's disease is the progressive disease with memory loss. It tops the list of neurodegenerative diseases affecting more than 36.5 million people above 60 years of age worldwide.[3, 4] Available treatments for the disease are donepezil, rivastigmine, galantamine and memantine which shows side effects like diarrhoea, vomiting, nausea, fatigue, insomnia, loss of appetite, and weight loss. Till now, available treatments provide only symptomatic relief and also they fail to stop the progression of disease pathology. Thus, there is a need to find newer targets and molecules for treatment of the disease.

Neuroinflammation mediated neurodegeneration is due to microglial cells activation.[5] Neuroinflammation leads to generation of Aβ plaque, mitochondrial dysfunction and oxidative stress resulting in neurodegeneration and neuronal death.[6,7] Studies report the anti-inflammatory action of nicotinic acid in atherosclerosis through cytokine inhibition[8] in monocytes and macrophages through GPR109a receptor.[8] Thus, the proposed study was aimed to target inflammatory component of neurodegeneration through nicotinic acid acting through GPR109a receptor.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals used in this study were of analytical grade. Nicotinic acid (86097-144722) was procured from Sisco Research Laboratories Pvt. Ltd. Maharashtra, India.

Animals

Three months old inbred male Wistar rats (180-220g) were procured from Central Animal Research Facility, Manipal University and used for the study. Animals were maintained in the animal house at a temperature of 23±2ºC and 12 hourly light and dark cycle. Three animals were caged in sanitized polypropylene cages in each group and the bedding contained sterile paddy husk. They were provided with abundant food and water on a daily basis. The experimental protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal University (No. IEAC/KMC/74/2015) and was carried in accordance with the CPCSEA guidelines, government of India. All experiments were performed between 9:00 am and 4:30 pm.

Assessment and induction of dementia using aluminium chloride

Morris water maze experiment

Morris water maze experiment is used to assess the spatial learning of rats.[9, 10] The basic and earliest version

of water maze test consists of a circular pool filled with water. Water level is determined by the size of the platform and is kept 2 cm above the platform. The pool is divided into 4 equal quadrants, either the platform or the position of rat is fixed to one of the quadrants. When the platform is fixed, rat is put into each quadrant to assess their learning behaviour to find the platform based on the distal cues. To eliminate the quadrant effect, four trials are performed in a day from four different quadrants. Four days of acquisition trials are conducted to make the rats familiar with the environment and on 5th day, probe trial is conducted to assess the learning. In the present experiment, a black circular pool with a diameter of 130 cm was used and filled with water till 2 cm above the height of platform (Water maze pool and platform, Ugo Basile, Italy). Pool was divided into four quadrants, named A, B, C, and D. Platform was made undistinguishable from the background and was placed in the D quadrant. The area in which platform was kept was named island. A checkboard was used as the visual cue placed on the wall. A video camera connected to computer software (Any-maze Video-Tracking Software, Ugo Basile, Italy) was hanged on top of the pool to record the movements of the rats.

Spatial acquisition

Rats were given four trials daily for four days to learn to find the platform. On the first day of the trial, rat was put into 'A' quadrant and left for 60s. If the rat reached the platform, it was kept on it for other 30s, but if rat was not able to reach to the platform, it was guided to the platform and kept there for 30s. A gap of 30s was given to the rat before putting it into second quadrant. This procedure was continued for the next three days.

Probe and Retention trial

After 24 hrs of the last trial, probe trial was conducted (on day 5) to assess the learning ability of rats. Rat was kept in the B quadrant and it was taken out when it reached to the hidden platform. Different parameters were checked to assess the learning ability. Those were escape latency (time taken by rat to reach the hidden platform), total distance travelled to reach the platform, latency to D quadrant and mean speed.

Treatment regimen

After the probe trial on day 5, animals were randomised into six groups with 6 animals in each group based on the escape latency. On 6th day, twenty four hours after the probe trial, disease induction was started till 48th day. On 49th day, retention trial was conducted again to assess the memory. Retention trial was performed same as the probe trial and the parameters mentioned above were recorded. Treatment was started on 50th day till 65th day for 15 day as follows.

Group 1-Normal control (normal saline i.p. 6th- 65th day + vehicle (0.25% w/v carboxymethyl cellulose) p.o. from 50th-65th day);

Group 2-$AlCl_3$ control, [10 mg/kg $AlCl_3$, i.p. 6th - 65th day + vehicle (0.25% w/v carboxymethyl cellulose) p.o. from 50th -65th day];

Group 3-Donepezil, [10 mg/kg $AlCl_3$, i.p. 6th - 65th day + 1.5 mg/kg donepezil p.o. from 50th -65th day];

Group 4-nicotinic acid (50 mg/kg), [10 mg/kg $AlCl_3$, i.p. 6th - 65th day + 50 mg/kg nicotinic acid p.o. from 50th -65th day];

Group 5- nicotinic acid (100 mg/kg), [10 mg/kg $AlCl_3$, i.p. 6th - 65th day + 100 mg/kg nicotinic acid p.o. from 50th - 65th day];

Group 6-nicotinic acid (200 mg/kg), [10 mg/kg $AlCl_3$, i.p. 6th - 65th day + 200 mg/kg nicotinic acid p.o. from 50th - 65th day]

All groups except normal control were continued with $AlCl_3$ dose during the treatment period (Day 50 – 65). On 65thday, retention trial was conducted again to assess the behavioural parameters using software (Any-maze Video-Tracking Software, Ugo Basile, Italy) as mentioned above.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test to determine the significance of difference between the groups except in analysing behavioural parameters (escape latency, total distance, latency to first entry to D quadrant and mean speed) on 49th day of $AlCl_3$ administration, where t test was used and while assessing body weight where two-way analysis of variance was used to analyse the data followed by Bonferroni test. Data represented as Mean ± SEM, n=6 at $P < 0.05$.

RESULTS AND DISCUSSION

Assessment of behavioural parameters using Morris water maze

Animals in $AlCl_3$ control group showed significantly increased escape latency and the total distance travelled as compared with normal control group. However, $AlCl_3$ administration did not show any significant effect on the time taken by rats for their first entry to D quadrant and mean swimming speed as compared to control group (Figure 1).

Effect of nicotinic acid on escape latency (EL)

$AlCl_3$ control group retained (as on 49th day) the raised escape latency on 65th day, which was found statistically insignificant as compared to normal control group. Standard drug, donepezil treatment significantly reversed the raised EL as compared with $AlCl_3$ group. Nicotinic acid at a lower dose (50mg/kg) barely decreased the escape latency as compared to $AlCl_3$ control but at higher doses (100 and 200 mg/kg) found effective in reversing the raised EL to much lower extent, but insignificantly as compared to $ALCl_3$ group (Figure 2).

Effect of nicotinic acid on total distance (TD)

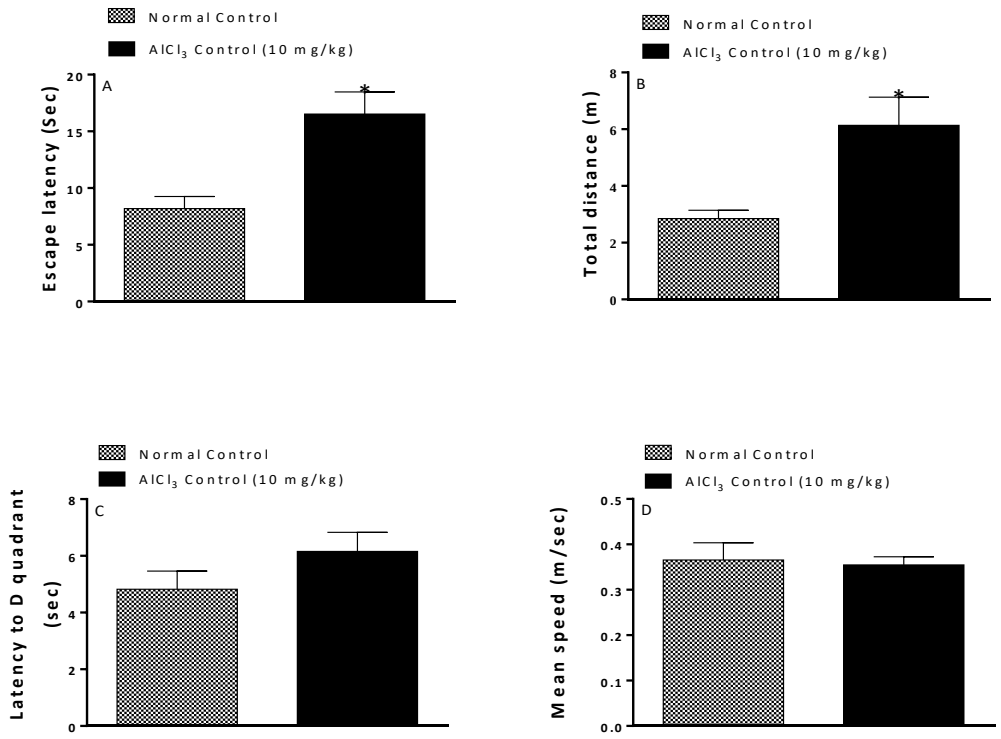
$AlCl_3$ control group insignificantly decreased the total distance as compared to normal control group. At 50, 100 and 200 mg/kg doses, nicotinic acid showed dose related effect on TD. As compared to $AlCl_3$ control, lower doses of nicotinic acid (50mg/kg and 100mg/kg) increased the distance travelled while donepezil and the higher dose of nicotinic acid (200mg/kg) decreased it insignificantly (Figure 3).

Effect of nicotinic acid on latency to D quadrant (LTD)

As compared to normal control, $AlCl_3$ control group showed increased LTD insignificantly. Donepezil reversed the $AlCl_3$ effect but not significantly. While nicotinic acid at a lower dose increased the latency, higher dose of 100 mg/kg did not show any change in LTD. Though at 200mg/kg, nicotinic acid was able to decrease LTD, it was found insignificant as compared to $AlCl_3$ control (Figure 4).

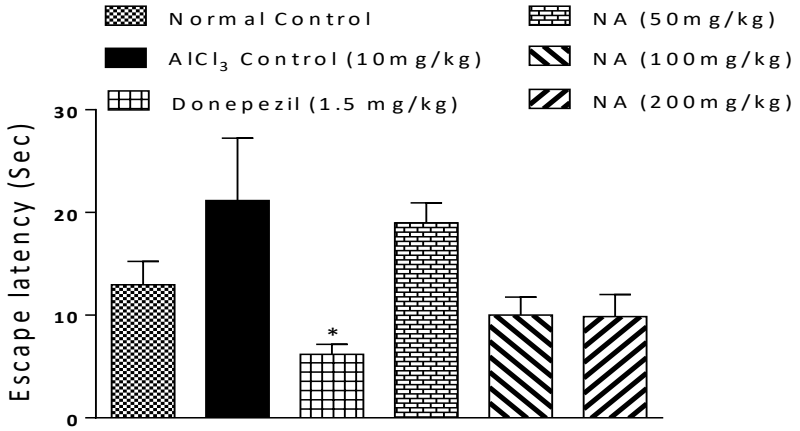
Effect of nicotinic acid on mean speed

None of the treatment showed any significant effect on mean speed of animals. However, $AlCl_3$ control and donepezil group exhibited a lower mean speed whereas nicotinic acid at all the doses showed higher mean speed comparative to $AlCl_3$ (Figure 5).



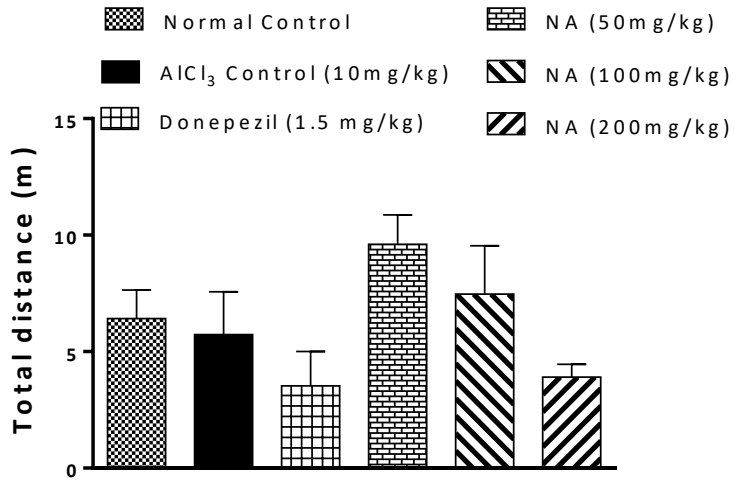
Data represented as Mean ± SEM at *P < 0.05.

Figure 1: Effect of $AlCl_3$ administration on A) escape latency, B) total distance, C) latency to D quadrant and D) mean speed.



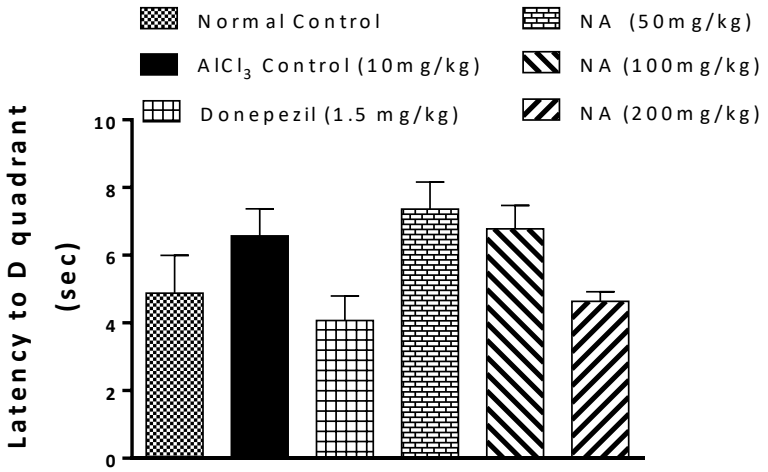
Data represented as Mean ± SEM, n=6. * represents P < 0.05 as compared to $AlCl_3$ Control

Figure.2: Effect of donepezil and nicotinic acid (NA) on EL in $AlCl_3$-induced dementia in rats.



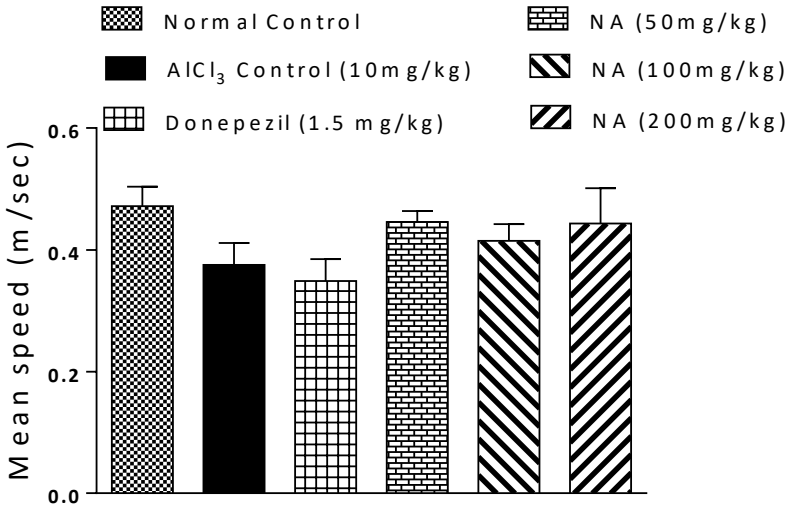
Data represented as Mean ± SEM, n=6.

Figure 3: Effect of donepezil and nicotinic acid (NA) on TD in $AlCl_3$-induced dementia in rats.



Data represented as Mean ± SEM, n=6.

Figure 4: Effect of donepezil and nicotinic acid (NA) on LTD in $AlCl_3$-induced dementia in rats.



Data represented as Mean ± SEM, n=6.

Figure 5: Effect of donepezil and nicotinic acid (NA) on mean speed in $AlCl_3$-induced dementia in rats.

CONCLUSION

In the present study, nicotinic acid was tested for its possible effect on cognitive deficit in rats. Aluminium chloride, a well-studied neurotoxin, was used to induce dementia in rats as discussed earlier. The protocol used in this study was standardized and reported earlier from our lab.[10-12] Learning ability of animals was assessed using escape latency, total distance, latency to first entry to D quadrant and mean speed in Morris water maze test. $AlCl_3$ administration significantly increased the escape latency and total distance on 49th day of administration which was not found consistent on 65th day of administration. After 15 days treatment, nicotinic acid (50, 100, 200mg/kg) did not show any significant effect on the behavioural parameters (EL, TD, LTD and mean speed). However, at higher dose i.e. 200 mg/kg, nicotinic acid was able to reverse the elevated escape latency, total distance and latency to D quadrant. No toxic effect of $AlCl_3$ and drug was observed on mean speed of animals. This suggest the normal health of animals after drug or toxicant administration. These results are in favour of possible therapeutic potential of nicotinic acid in dementia. However, a detailed investigating with in vitro and in vivo studies will help to produce the conclusive results for assessing the modulatory role of GPR109a receptor agonist against neurodegeneration.

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