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Neuro-protective effect of Suramin against aluminum-induced cognitive dysfunction in rats

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Abstract

Suramin is N-methyl-D-aspartate (NMDA) receptor antagonist which protects against the glutamate excitotoxicity and oxidative stress. Aluminum is a potent neurotoxin involved in the initiation and progression of various cognitive disorders like Alzheimer's disease (AD). Chronic aluminum exposure induces glutamate excitotoxicity, oxidative stress and increases amyloid beta levels *in vivo*. Therefore, the present study was designed to explore the possible role of suramin against aluminum mediating cognitive dysfunction in rats. Aluminum chloride (100 mg/kg, i.p.) was given to rats daily for 30 days to induce cognitive dysfunction. Suramin (25, 50 and 100 mg/kg, i.v.) and physostigmine (0.50 mg/ kg i.p.) was given for 30 days along with aluminium treatment. On the 31st day of the study, various behavioral tests (radial arm maze and elevated plus maze task paradigms) were done to evaluate cognitive tasks. According to the study chronic aluminum chloride administration resulted in poor retention of memory in radial arm maze and elevated plus maze task paradigms. Chronic administration of suramin significantly improved memory retention tasks in aluminum-treated rats. The study advocates a cognitive enhancing effect of suramin against aluminum-induced cognitive dysfunction.

Keywords: Alzheimer's disease, Excitotoxicity, Suramin, physostigmine.

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1. Introduction

The disturbances of the intracellular ionic homeostasis after activation of channel-associated membrane receptors by the excitatory neurotransmitters represents a principle event that triggers excitotoxic cell death of neurons. Excitotoxicity plays an important role in a number of neuropathological patterns and is regarded as a key player in neurodegeneration during ischemia, trauma, and chronic neurological disorders [1]. Therefore, the search for therapeutic drugs, which prevent or attenuate ion-dependent excitotoxicity, has an important value for a range of neurological disorders.

Suramin (Sur) is a polyanionic chemotherapeutic agent, which has been used for a long time in the therapy of Rhodesian trypanosomiasis[2].

Currently, this drug has been clinically tested for a variety of human cancers. The mechanism of therapeutic activity of suramin is poorly understood. Interference with growth factor receptor function and induction of lysosomal storage defects may contribute to the cytostatic and antineoplastic activity of the drug. In addition to the interference with the activity of several lysosomal enzymes, suramin is able to inhibit protein kinase C and topoisomerase II the enzymes that are critically involved in the control of cell growth and proliferation [3-7]. Besides antineoplastic, suramin has also a marked antiviral activity capable of inhibiting reverse transcriptase of a number of retroviruses [8]. Recently, the antagonistic effect of suramin has been extended to the g-aminobutyric acid (GABA), and glutamate receptor channels. Suramin has also been suggested for its antioxidant effect and neuroprotective effect against

excitotoxic cell death [9]. Based on this, the present study was designed to investigate the neuroprotective effect of suramin against aluminum-induced cognitive impairment in rats.

2. Materials and Methods

2.1 Chemical:

Aluminium chloride was procured from Hi-Media, India. Suramin was procured from MP Biomedicals, USA. Aluminium chloride ($AlCl_3$) and suramin solutions were made freshly at the beginning of each experiment. For i.p. administration, aluminum chloride was dissolved in sterile water; suramin and physostigmine (Physo) were dissolved in 0.9% saline solution.

2. Experimental Animals:

Healthy adult male albino rats of Wistar strain weighing (200-230 g) were used from the Institute of Pharmaceutical Sciences and Research, Wardha. The animals were acclimatized to the standard laboratory conditions with temperature (25±2°C) and fed with standard animal pellet feed (Hindustan lever limited), *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) with approval no: (535/02/a/CPCSEA) for the care and use of animals.

Total 36 animals divided into six groups each containing 6 animals. Animals were randomized based on their body weight:

Group I (Saline): Rats were administered saline solution intraperitoneally (i.p.) for 30 days.

Group II ($AlCl_3$): Rats were administered aluminum chloride (100 mg/kg i.p.) for 30 days.

Group III ($AlCl_3$ + Physo): Rats were administered intraperitoneally with 0.50 mg/kg of physiostigmine daily for 30 days.

Group IV ($AlCl_3$ + Sur 25 mg/kg): Rats were administered with suramin 25 mg/kg intravenously on 1,8,16, and 24th day during aluminum chloride treatment.

Group V ($AlCl_3$ + Sur 50 mg/kg): Rats were administered with suramin 50 mg/kg intravenously on 1,8,16, and 24th day during aluminum chloride treatment.

Group VI ($AlCl_3$ + Sur 100 mg/kg): Rats were administered with suramin 100 mg/kg intravenously on 1, 8, 16, and 24th day during aluminum chloride treatment.

The doses of suramin and aluminum chloride and physostigmine were selected on the basis of those reported in the literature [10,11]. The study lasted 31 days.

2.3 Behavioral assessments

2.3.1 Radial arm maze test

The test was conducted in an eight-arm maze made of wood, in which the arms were extended from an octagonal center compartment with 35 cm diameter. Each arm of the maze was 56 cm long and 10 cm wide with 10 cm high rails along the length of the arm. The maze was well illuminated and numerous cues were present. Arms were baited with Food pellets (reward) in grooves that were located 2 cm at the end of the arms. During the test, rats are fed once a day and their body weights maintained at 85% of their free-feeding weight to motivate the rat to run the maze. Animals were trained on a daily basis in the maze to collect the food pellets. All groups of animals were trained so that they would become habituated to the apparatus and food pellets for 3 days before the actual training. Rats were shaped to run to the ends of the radiating arms and the baits were gradually restricted to grooves. During this 3-day period, a 10-min period of habituation was repeated 3 times a day, at intervals of more than one hour. In each training session, the rat was placed in the central area of the 8-arm radial maze facing arm one, timing had begun rat was free to explore in the maze. The trial was judged complete when the rat chose all baited arms or had spent 10 min. No arm was rebaited after the testing began [12].

The performance of a given animal in each trial was assessed using three parameters:

1) The number of correct choices (number of correct arm entries is the number of entries until an error was made),
2) The number of errors which was defined as choosing arms which had already been visited, and
3) The time elapsed before the animal ate all 8 pellets.

The animals (before starting the treatment) who made 7 or 8 correct choices and less than one error in three successive sessions, they were then used in the evaluation.

The experimentwas conducted in order to test the chronic effect of drug treatment. For the experiment, rats were kept on a restricted food schedule for 30 days. During this period, chronic drug treatment was administered. Physostigmine was given daily and Suramin on 1, 8, 16, 24 days and the animals were trained before the end of treatment for the last three days and reading was taken after the last dose (31st day) [13,14].

2.3.2 Elevated plus maze paradigm

The elevated plus maze consisted of two opposite black open arms (50 x 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10 x 10 cm and the entire maze was placed 50 cm above the ground. Acquisition of memory was tested on day 30 from the start of aluminum chloride administration. Rats were placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as the transfer latency. Animals were allowed to explore the maze for 30 sec. after recording the transfer latency and animals were then

returned to the home cages. If the animal did not enter the enclosed arm within 180 sec., it was pushed on the back into one of the enclosed arms and the transfer latency was recorded as 180 sec. Retention of memory was assessed by placing the rat in an open arm and the transfer latency was noted on day 31st. [15,16]

2.4 Statistical Analysis:

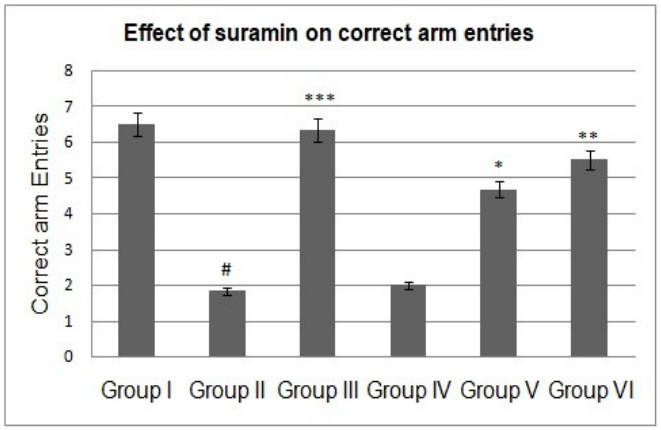
Data were analyzed using GraphPad Prism 5 for Windows (version 5.00). Results were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) and Dunnett's t-test was used to test the significance of the difference between the variables in various groups. The p values of less than 0.05 were considered to be statistically significant.

3. Results

3.1 Effect of suramin on correct arm entries

Figure 1 shows the effect of suramin on correct arm entries in radial arm maze. Aluminium chloride treatment significantly [$F_{(5, 30)} = 8.85, p < 0.001$] decreased the number of correct arm entries compared to normal. There was a significant increase in correct arm entries by treatment of suramin (50 mg/kg, 100 mg/kg) and physostigmine (0.50 mg/kg) as compared to control. Effect of suramin (100 mg/kg) was comparable to standard drug physostigmine.

Figure 1: Effect of suramin on correct arm entries

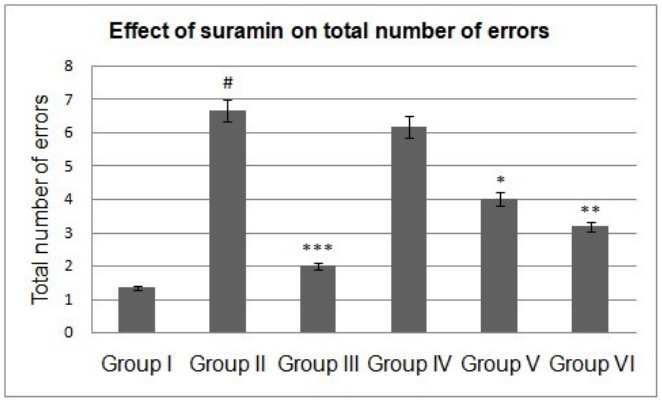


# p< 0.001 compared to normal and (*** p <0.001, ** p < 0.01, * p < 0.05) compared to control

3.2 Effect of suramin on total number of errors

Figure 2 shows the effect of suramin on a total number of errors in radial arm maze. Aluminium chloride treatment significantly [$F_{(5, 30)} = 10.05, p < 0.001$] increased the total number of errors as compared to normal. There was a significant decrease in the total number of errors by treatment of suramin (50 mg/kg & 100 mg/kg) and physostigmine (0.50 mg/kg) as compared to control. Effect of suramin (100 mg/kg) was comparable to standard drug physostigmine.

Figure 2: Effect of suramin on total number of errors

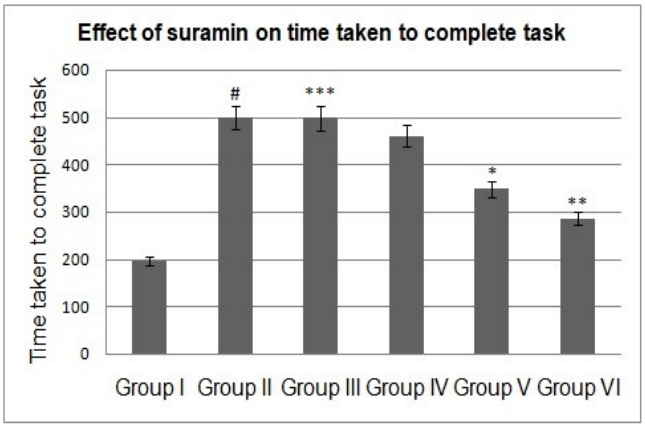


# p< 0.001 compared to normal and *** p < 0.001, ** p < 0.01* p < 0.05 compared to control.

3.3 Effect of suramin on time taken to complete task

Figure 3 shows the effect of suramin on time taken to complete the task in radial arm maze. Aluminium chloride treatment significantly [$F_{(5, 30)} = 11.54, p < 0.001$] increased the time taken to complete the task as compared to normal. There was a significant decrease in time taken to complete the task by treatment of suramin (100 mg/kg, 50 mg/kg) and physostigmine (0.50 mg/kg) as compared to control. Effect of suramin (100 mg/kg) was comparable to standard drug physostigmine.

Figure 3: Effect of suramin on time taken to complete task



# p< 0.001 compared to normal and *** p < 0.001, ** p < 0.01 & * p < 0.05 compared to control.

3.4 Elevated plus maze performances

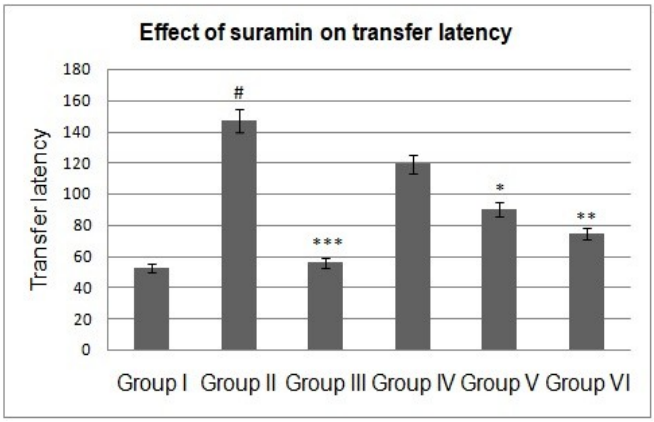
Effect of suramin on memory performance in elevated plus maze task paradigm in aluminium chloride-treated rats

3.5 Effect of suramin on transfer latency

Figure 4 shows the effect of suramin on transfer latency in elevated plus maze. Aluminium chloride Treatment significantly [F (5, 30) =6.56, p < 0.001) increased the

transfer latency as compared to normal. There was a significant decrease in the transfer latency by the treatment of suramin (50 mg/kg & 100 mg/kg) and physostigmine (0. 50 mg/kg) as compared to control. Effect of suramin (100 mg) was comparable to standard drug physostigmine.

Figure 4: Effect of suramin on transfer latency



#p < 0.001 compared to normal and *** p < 0.001, ** p < 0.01 & * p < 0.05 compared to control.

4. Discussion

Alzheimer's disease (AD) is a neurodegenerative disorder associated with a decline in cognitive abilities. The hallmark of AD is the neuronal degeneration associated with senile plaques, neurofibrillary tangles which are composed of amyloid β-peptide (Aβ). The deposition of soluble Aβ produces the aggregation of the peptide forming amyloid fibrils which have been reported to be neurotoxic *in vitro* and *in vivo*. Patient with Alzheimer's disease become bed-bound and reliant on 24/7 care and ultimately leads to death.

Despite the severity and high prevalence of this disease, the allopathic system of medicine is yet to provide a satisfactory antidote.[17] Hence, the present study focuses on the exploration of the memory-enhancing activity of the suramin in an aluminum chloride induced amnesia rat model.

Experimentally, it has been demonstrated that chronic aluminum exposure causes neurological signs and results in intra-neuronal neuro-filamentous aggregation of proteins akin to neurofibrillary tangles (in the hippocampus, cerebral cortex, brain stem and spinal cord) and biochemical changes as seen in patients with cognitive disorders. Upon entering the brain through the blood-brain barrier, aluminum can interact with various enzyme pathways of the brain. Aluminum causes progressive deterioration of mitochondrial functions due to excessive free radical generation. This further damages other cellular molecules including DNA damage, nitration of protein residues and lipid peroxidation[18].

A report has demonstrated that aluminum supplementation causes an increase in acetylcholinesterase level in the brain. Aluminum is a potent cholinotoxin and causes apoptotic neuronal loss which is a characteristic symptom of Alzheimer's disease. Chronic aluminum treatment increases glutamate level in the brain in various ways. Glutamate levels in the brain may also be modulated by its altered metabolism. Aluminum causes marked oxidative damage by increasing the redox-active iron concentration in the brain mainly via the Fenton reaction. At the molecular level, it influences DNA topology, gene transcription, and cellular energy metabolism. It induces misfolding and self-aggregation of highly phosphorylated cytoskeleton proteins such as neurofilaments or microtubule-associated proteins and Aβ which are implicated in Alzheimer's disease.[19] Aluminium-induced Alzheimer's disease is a well-documented model of experimental Alzheimer's disease.

In the present study we investigated the behavioral changes caused by chronic aluminium exposure and the possible effect of suramin treatment using two behavioral tests; radial arm maze and elevated plus maze.

Radial arm maze has been extensively used in learning and memory studies and has served as the basic task for one of the most important theories on the role of the hippocampus in learning & memory. The rat uses spatial information provided by the distal cues in the room to efficiently locate the baited arms (Arms with food pellet). The performance of eachratwas assessed for the number of correct choices, the total number of errors and time taken to complete the task. [20]

Radial arm maze performance in aluminum treated rats was severely impaired as compared with untreated (normal) rats, confirming earlier findings. The present findings indicate that the impaired performance of aluminum treated rats is related to cognitive impairment since the performance of trained aluminum treated rats was poor as compared to normal in the RAM task. Similarly, elevated plus-maze performance in aluminum treated rats was also severely impaired. This test also revealed that aluminum reduced learning and memory performance.

In the radial arm maze performances, it is found that suramin (100 mg/kg) produces significant improvement in memory performances comparable that of standard drug Physostigmine ($P < 0.001$). Suramin (100 mg/kg) significantly increased ($P < 0.01$) the correct arm entries (Figure 1), decreased the total number of errors (Figure 2) and time to complete the task (Figure 3) in Radial Arm maze (RAM) as compared to control group.

Suramin (50 mg/kg) group was also found to increase significantly ($p < 0.05$) the correct arm entries (Figure 1), decreased the total number of errors (Figure 2) and time to complete the task (Figure 3) in RAM as compared to control (aluminium chloride treated) group but not as significant ($P < 0.01$) as suramin (100 mg/kg).

Suramin (25 mg/kg) was found to be having non-significant (Figure 1, 2 and 3) effect on all above-mentioned parameters in RAM.

Additional evidence for the memory-enhancing effect of the suramin was obtained from the studies on the elevated plus maze. The Elevated Plus Maze (EPM) test is suggested to be a simple method for the evaluation of learning and memory processes. Since the animals are able to remember the configuration of the open and enclosed arms, they escape from the unsafe open arm more rapidly on the second trial. It is possible to evaluate fear-motivated learning, which underlies the transfer latency procedure in this test [21]. In EPM, aluminum exposure was associated with an increase transfer latencies (figure 4) as shown by the significant increase in the time required to enter in the enclosed arm where as suramin treated rats show a decrease in transfer latency in elevated plus maze. In the presentstudy, the shortened transfer latency was obtained on the retention phase, i.e. on 31st day in treated groups and the effect was statistically significant.

Suramin (100 mg/kg) group was found to produce significant improvement in memory performances ($P < 0.01$) in EPM. Suramin (50 mg/kg) group shortened transfer latency but not as significant ($P < 0.01$) as suramin (100 mg/kg). Suramin (25 mg/kg) was found to be non-significant as compared to control (aluminium treated) group. (Figure 4)
This study consolidates that chronic administration of suramin 100 mg/kg improves memory performances of rats in radial arm maze and elevated plus maze.

It is well known that glutaminergic systems play an important role in cognitive deficits. One is the possibility that exogenous glutamate, or related compounds acting on glutamate receptors, can be consumed in the diet and damage the brain. Second, there is the possibility that endogenous glutamate released from neurons can contribute to acute neuro-degeneration occurring in relation to cerebral ischemia or traumatic brain injury. Third, there is the possibility that activation of glutamate receptors contributes to the process of cell death in chronic neurodegenerative disorders, such as motor neuron disease or amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease. [22] From the present study, it can be stated that aluminum showed significant impairment of memory as there higher incorrect arm entries, increased number of errors, the timeis taken to complete the task and shorten transfer latency in RAM and EPM respectively. Treatment with chronic suramin restored memory impairment by $AlCl_3$. Suramin might have protected aluminum-induced cognitive dysfunction by reducing glutamate excitotoxicity and oxidative stress in memory-impaired rats. The present study for the first time reports suramin as a cognitive enhancer for aluminum-induced memory impairment in rats.

4. Conclusion

Suramin is a cognitive enhancer against $AlCl_3$ induced memory impairment. Suramin treated rats showa statically significant increase in the number of correct arm entries, reduced total number of error; they ended their missions faster than non-treated animals in RAM and shortened transfer latency in EPM. In light of the above data, it may be worthwhile to explore the potential of this drug in the management of cognitive dysfunction.

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