

Amelioration of Motor and Non-Motor Deficits by Agmatine-Loaded Solid Lipid Nanoparticles in a 6-OHDA Rat Model of Neurodegeneration

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Abstract

Neurodegenerative diseases are characterized by the progressive loss of neurons, leading to profound impairments in cognitive and motor functions. Conventional medical interventions often fail to impede disease progression and frequently result in enduring adverse effects. This study examined the neuroprotective properties of agmatine and its solid lipid nanoparticle (SLN) formulation in an animal model of induced neurodegeneration. Exposure to neurotoxic substances resulted in significant behavioral alterations. The neurotoxicant group exhibited substantial spatial learning impairments, evidenced by a prolonged escape latency in the Morris Water Maze (74.33 ± 0.70 seconds). Agmatine SLNs demonstrated superior neuroprotective efficacy compared to free agmatine. In the Elevated Plus Maze (EPM), agmatine SLNs significantly improved cognitive recovery by normalizing transfer latency to 59.13 ± 0.78 seconds, which was nearly identical to the normal control value (58.22 ± 0.84 seconds). Furthermore, while the neurotoxicant group showed significant motor impairment with only 185.3 ± 13.05 counts/5 min in locomotor activity, the agmatine SLN-treated group maintained significantly higher activity (270.3 ± 22.36 counts/5 min). These findings suggest that agmatine SLNs effectively ameliorate both motor and non-motor symptoms, offering a promising therapeutic strategy for progressive neuronal degeneration.

Keywords: Levodopa, Agmatine, 6-hydroxydopamine (6-OHDA), Solid Lipid Nanoparticles, Intracerebroventricular Injection, Neurodegeneration

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1. Introduction

Neurodegenerative diseases represent one of the most significant and growing global health challenges of the 21st century. As the global population ages, the prevalence of conditions such as Parkinson's disease (PD), Alzheimer's disease (AD), and Amyotrophic Lateral Sclerosis (ALS) is rising at an alarming rate. These disorders are characterized by the progressive and irreversible loss of specific neuronal populations within the brain and spinal cord. Unlike many other medical conditions where tissues can regenerate or compensate for damage, the central nervous system (CNS) possesses limited regenerative capacity[1]. Consequently, once a threshold of neuronal death is reached, the functional deficits become permanent and increasingly severe[2].

The clinical presentation of neurodegeneration is multifaceted, typically manifesting as a combination of debilitating motor impairments and pronounced non-motor symptoms. Motor deficits often include tremors, rigidity, bradykinesia (slowness of movement), and postural instability[3]. However, it is the non-motor symptoms—such as cognitive decline, anxiety, depression, sleep disturbances, and apathy—that often pose the greatest burden on the quality of life for both patients and caregivers. Despite decades of intensive research, the medical community still lacks definitive

cures[4]. Current pharmacological interventions, such as Levodopa for Parkinson's, primarily offer symptomatic relief. While these treatments can manage symptoms in the early stages, they fail to halt or even slow the underlying disease progression. Over time, their efficacy often wanes, and patients frequently experience "off-periods" or severe long-term side effects like dyskinesia[5], [6].

The underlying pathophysiology of neurodegeneration is complex and involves a cascade of interconnected cellular events. Key hallmarks include chronic neuroinflammation, the accumulation of misfolded protein aggregates (such as alpha-synuclein or beta-amyloid), and persistent oxidative stress. Oxidative stress, in particular, plays a central role; it occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms[7]. In a neurodegenerative state, vital antioxidants like glutathione (GSH) are depleted, leading to lipid peroxidation and the subsequent destruction of neuronal membranes[8]. This cellular environment creates a "vicious cycle" where inflammation triggers more oxidative stress, which in turn leads to further protein misfolding and neuronal death. In the specific context of Parkinsonism, the loss of dopaminergic neurons within the nigrostriatal system

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is the primary driver of motor dysfunction. The nigrostriatal pathway is essential for the coordination of smooth, purposeful movement; its disintegration leads to a critical deficiency of dopamine, resulting in the classic motor symptoms of tremors and balance problems[9]. Simultaneously, the degradation of the basal forebrain complex and other non-dopaminergic pathways contributes to the non-motor spectrum of the disease. Cognitive impairments and dementia arise as the cholinergic and serotonergic systems are compromised. Addressing this dual burden of motor and non-motor deficits requires a therapeutic agent that can address multiple pathological pathways simultaneously[10].

Agmatine, an endogenous polyamine derived from L-arginine by the enzyme arginine decarboxylase, has emerged as a highly promising neuroprotective candidate[11]. Agmatine is often referred to as a "novel neuromodulator" due to its diverse range of biological activities. It acts as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, which is crucial for preventing glutamate-induced excitotoxicity—a major cause of neuronal death. Furthermore, agmatine has demonstrated powerful antioxidant properties and the ability to reduce the production of pro-inflammatory cytokines such as TNF- α and IL-6. By modulating these pathways, agmatine can potentially shield neurons from the toxic environment found in neurodegenerative diseases. However, the clinical application of agmatine faces a significant hurdle: its pharmacokinetics. Agmatine possesses poor oral bioavailability and, perhaps more importantly, it cannot efficiently cross the blood-brain barrier (BBB)[12]. The BBB is a highly selective semipermeable border that protects the brain from harmful substances but also prevents over 98% of small-molecule drugs from reaching their target in the CNS. To realize the therapeutic potential of agmatine, a specialized delivery system is required that can bypass or penetrate this barrier effectively[13], [14].

To overcome these challenges, this study utilizes Solid Lipid Nanoparticles (SLNs) for targeted brain delivery. SLNs are an innovative drug delivery system composed

of a solid lipid core that can encapsulate lipophilic or hydrophilic drugs[15]. They offer several advantages, including high biocompatibility, the ability to protect the encapsulated drug from chemical degradation, and, crucially, the ability to cross the blood-brain barrier via various transport mechanisms. By encapsulating agmatine within SLNs, we aim to enhance its concentration in the brain, thereby maximizing its neuroprotective effects while minimizing systemic side effects[16].

This research employs the 6-hydroxydopamine (6-OHDA) rat model, a well-established experimental tool for mimicking the selective catecholaminergic depletion seen in neurodegeneration[17]. By administering 6-OHDA, we can induce specific motor and non-motor deficits that allow for the rigorous testing of therapeutic interventions[18]. This study compares the efficacy of free agmatine against its encapsulated SLN formulation. The primary objective is to demonstrate that agmatine-loaded SLNs can significantly ameliorate both motor impairments and cognitive/behavioral deficits. By focusing on behavior-based parameters, this research seeks to provide a foundation for future disease-modifying therapies that improve the functional lives of those suffering from neurodegenerative disorders[19].

2. Material and methods:

2.1. Animals

Male Wistar rats weighing 280 \pm 10 g were acquired from the institute's animal house. The animals were fed and given water in typical husbandry conditions. At 21 \pm 3 °C, the temperature was kept constant. In accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry, and Dairying, Government of India, New Delhi, the experimental protocol was approved by the Institutional Animal Ethics Committee (Ph.D.DO1222005/KLECOPH/23)[20], [21].

Five groups of six animals each were formed from the animals. They are as follows (Table 1):

Group No.	Group Description	Number of Animals
1	Normal Control	6
2	Neurotoxicant Control (e.g., 6-OHDA)	6
3	6-OHDA + Free Agmatine (50 mg/kg)	6
4	6-OHDA + Agmatine Eposomes (50 mg/kg)	6
5	6-OHDA + Std drug Levodopa (0.5 mg/kg)	6
	Total	30

Table 1. Grouping of experimental animals (n=6)

i. Drug administration

Single dose of 20 μ g of 6 - hydroxy dopamine (6-OHDA) is administered by intracerebro ventricular

(*i.c.v*) injection at Striatum of brain. The Levodopa (0.5 mg/kg) as a standard drug was administered orally[22].

2.2. Assessment of behavioural activity

2.2.1 Elevated plus maze

In rat models of Parkinson's disease (PD) with lesions brought on by 6-OHDA, anxiety-like behaviour—a common non-motor symptom—is assessed using the Elevated Plus Maze[23]. With two open arms and two closed arms (about 50×10 cm), the device is situated between 50 and 70 cm above the ground[24]. The closed arms' walls are between 30 and 40 cm high. Rats require a minimum of 30 minutes to acclimate to the testing environment[25]. Each rat is carefully placed with its arm open on the center platform on test day, and it is allowed to explore for five minutes[26]. The main metrics used for measurement are the number of entries into open and closed arms (with all four paws inside an arm), the percentage of entry into open arms, and the amount of time spent in both open and closed arms. Reduced open-arm exploration is a sign of increased anxiety-like behaviour, which is common in PD models[27]. To differentiate between anxiety and reduced mobility, results must be analysed with motor activity controls (e.g., closed-arm entries or the open-field test), considering that PD rats may exhibit locomotor deficits[28], [29].

2.2.2. Morris water maze (MWM)

The MWM assesses memory and spatial learning. The animals were trained on a platform in a circular pool with a diameter of 180 cm and a depth of 60 cm[30]. Four equal quadrants were created out of the pool (Q1, Q2, Q3, and Q4). The starting point for each trial was the same—one of the quadrants[31]. The pool was filled with water to a depth of 40 cm and stored in the darkroom. To analyse escape latency time (ELT), a movable platform was positioned in a pool with water level below 2 cm. ELT, or the amount of time it took the animals to find the concealed platform, was measured after they were educated for eight to eleven days[32], [33]. This provided an index of learning. The rats were given two minutes to swim in search of the platform; if they were unsuccessful, they were led onto it and given the option to stay there for twenty seconds. The platform was taken down on the twelfth day, and rats were given ninety seconds to investigate it[34]. The index of retrieval was obtained by recording the amount of time spent in the target quadrant (TSTQ).

2.2.3. Light and dark model

A typical non-motor symptom of mice models of Parkinson's disease (PD), particularly those brought on by 6-OHDA or MPTP, is anxiety-like behaviour, which is assessed by the Light-Dark Box test. The apparatus consists of two elements: a brilliant, accessible light chamber approximately 27×27 centimetres and a sealed, dim or unilluminated dark chamber measuring approximately 18×27 centimetres[35]. A tiny entryway links the two. At least half an hour prior to the experiment, the rodents are acclimated to the testing environment. Each rat is carefully positioned in the illuminated chamber, oriented away from the entrance,

and given five minutes to investigate its surroundings before the test begins. To eliminate odor signals, the apparatus is cleaned with a 10% ethanol solution in between animals[36].

A video-tracking device keeps an eye on and evaluates behaviour. The amount of time spent in both illuminated and darkened compartments, the frequency of transitions between these areas, and the latency to enter the shaded chamber are the main metrics that are assessed[37]. Less time spent in the light area, fewer compartment transitions, and a faster entry into the dark compartment are all signs of heightened anxiety-like behaviour in rat models of Parkinson's disease. Transition counts should be carefully interpreted and, if at all possible, complemented by motor evaluations like the open-field or rotarod test, given the potential motor deficiencies in PD models that could impair mobility[38].

2.2.4. Rotarod test

Using rotarod equipment (Orchid Scientifics, Nashik, Maharashtra), the muscle coordination was captured. The mice's capacity to stay on a rotating rod is assessed in this experiment (Rozas and Garcia, 1997[39]). In this experiment, the speed of the rotarod was fixed to 30 RPM and time was fixed at two min. The final evaluation was conducted on the fourteenth day after all the animals had been taught for six days to achieve stable performance. On the rotarod, the average retention time was computed[40].

2.2.5. Locomotor activity

The locomotor activity was analysed by digital actophotometer (INCO Photo-Actometer, Instruments and Chemicals Pvt. Ltd, Ambala, India). The actophotometer is made up of photocells that are sensitive to infrared light in a closed arena. The animals were housed in a closed arena and watched for five minutes[41]. The light beam in the actophotometer cut or crossed by the animals was recorded and expressed as counts per 5min. To prevent outside disturbances that could impact the animal's movement, the equipment was housed in a dark, sound-attenuated, and ventilated chamber[42].

2.2.6. Passive avoidance test

In rat models of Parkinson's disease (PD), such as those produced by 6-OHDA or MPTP, researchers employ the Passive Avoidance test to assess memory and learning deficits[43]. The device consists of a two-sectioned container with a brilliantly lit part and a dimly lit area with a grid-like floor attached to a shock generator. Before the training experiment in the light chamber starts, each rat spends half an hour acclimating to the testing room. After all four paws have entered the dark room, the door is secured, and a brief electrical shock (0.3–0.5 mA for 1-2 seconds) is administered[44]. The rat is reintroduced to the light box without being shocked in order to do the retention test, which is typically carried out 24 hours later. The step-through delay to enter the dark room is then measured, with a cut-off of around 300

seconds. Models of Parkinson's disease frequently exhibit memory issues, such as decreased latency. When assessing the results, any motor anomalies must be taken into account[45].

2.2.7. Open field test

The arena's nine squares make up the open field. The mice were positioned in the corner, facing out, and given five minutes to roam around. It was noted how many canter zones were reached and how many lines were crossed with both forepaws. After every test, 70% ethanol was used to clean the equipment. During the experiment, the video was captured, and it was scored[46].

2.2.8. Beam walk test

The beam walk test was used to assess balance and motor coordination. The mice were permitted to travel 50 cm between platforms on a wooden beam[47]. To keep the fallen animals safe, a cushion was placed beneath the beam. Tests were conducted 24 hours after the animals were trained on the 12th day. The number of slips off the beam was counted during the one-minute test to accomplish the target[48]. A seven-point rating system was used to evaluate the foot slips. 0: The mice were unable to remain; 1: The mice remained on the beam without moving; Two, the mice attempted to move but fell off; three, they crossed the beam with several slips (4-6); four, they crossed the beam with several slips (2-3); five, they crossed the beam with a single slip; and six, they crossed the beam without any slips on their hindlimbs[49], [50].

2.2.9. Cylinder test

A cylinder test was used to assess the motor deficiencies in the left forelimb. It evaluates the mice's free will movement inside a clear glass cylinder. The animals were put inside the clear cylinder, and during five minutes, the number of contacts each forelimb and both forelimbs made with the cylinder wall was counted. The formula $[(\text{contralateral side} + 1/2 \text{ both}) / (\text{ipsilateral side} + \text{contralateral side} + \text{both})]$ was used to determine the score. A score of 0.5 shows that forelimbs were employed with the same frequency, while a ratio of less than 0.5 suggests a motor deficiency in the contralateral forelimb[51].

2.5. Statistical analysis

Every experiment was conducted in triplicate, and the mean \pm standard deviation was used to present the results.

Tukey's Test was used to compare the means after an ANOVA was completed. A value of $P < 0.05$ was deemed statistically significant[52].

3. Results and Discussion

3.1. Elevated Plus Maze

Transfer latency increase after 6-OHDA administration impairs the acquisition of learning and consolidation of memory. The modified Elevated plus Maze is an ideal paradigm to assess cognition as it shows impaired exposure of animals to the open arm, through a drug-induced delay of transfer latency that leads to antisocial behavior linked with hippocampal and corticostriatal deficits. Thus, dopaminergic neurotoxicity critically disrupts neural circuits underlying cognitive processing, as evidenced by a marked increase from 58.22 ± 0.84 s for the normal control group to 83.43 ± 0.80 s for the 6-OHDA group (Fig. 1) [53]. This is consistent with research showing that dopaminergic loss preferentially impacts relay areas responsible for memory (i.e. hippocampus, prefrontal cortex) and motor pathways 1. The transfer latency (in seconds) was significantly decreased to 65.27 ± 0.65 in native agmatine administration compared with nontransferred stage of paired publication session that occurred in this period of time with high concentration at the third moon (Table 2). This enhancement implies that, potentially through reducing oxidative stress, inhibiting NMDA receptor-mediated excitotoxicity and modulating mater bioactive neuro-inflammatory mediators agmatine preserve neurons[54]. But the values were still proportionately very different from normal control. Since the normalization is incomplete, it's likely that it cannot even enter the brain or be utilized by its body when needed. By contrast, agmatine-loaded SLN almost fully prevented cognitive impairment; transfer latency (59.13 ± 0.78 s) was close to normal and not significantly different from that of the control group. There near (that is, above 80%) normalisation of level was a mechanistic feature that also correlates systemically to SLN delivery sustains therapeutic range of agmatine and help access to central nervous system. These data, in addition to demonstrating the therapeutic equivalence of the SLN formulation to standard treatment provide compelling evidence for its translational potential. The benefits of agmatine in restoring cognition and memory impairment induced with 6-OHDA are significantly greater with nanoencapsulation compared to traditional delivery methods[55].

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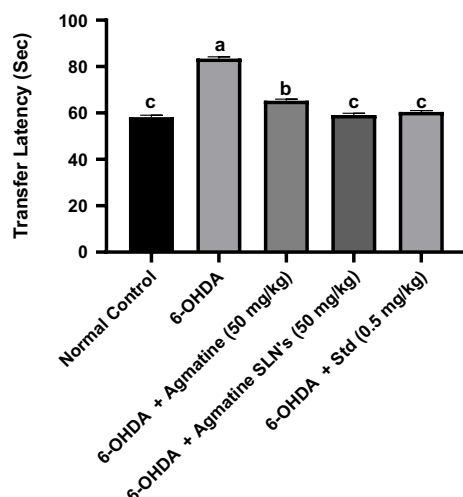


Figure 1 Agmatine SLNs' impact on learning and memory impairments caused by EPM in experimental rats with 6-OHDA-induced neurodegeneration. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table 2. Effect of Agmatine SLN's on memory and learning deficits by EPM on 6-OHDA induced neuro degeneration in experimental rats[56].

S. No.	Groups	Transfer Latency (Sec)
1.	Normal Control	58.22 ± 0.84 ^c
2.	Neurotoxicant Control (e.g., 6-OHDA)	83.43 ± 0.80 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	65.27 ± 0.65 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	59.13 ± 0.78 ^c
5.	6-OHDA + Std (0.5 mg/kg)	60.42 ± 0.60 ^c

3.2. Morris Water Maze Test:

The evident cognitive impairment with administration of 6-OHDA is reiterated by the Morris Water Maze results. For the escape latency, the neurotoxicant group (74.33 ± 0.70 s) was significantly increased compared with normal control (42.20 ± 0.50 s), indicating that spatial learning and memory acquisition were clearly disturbed in rats exposed to neurotoxicants (Fig. 2, Table 3) The task relies on spatial navigation, and thus on hippocampal integrity as well as the functional connectivity of dopaminergic and cortical systems. Notably, the observed delay following a 6-OHDA lesion suggests both rostral (dopaminergic)-caudal (hippocampally-related) dopaminergic neurodegeneration circuit as well as replicating dementia-defining non-motor deficits of progressive degenerative disorders.

Native agmatine's ability to improve learning and memory was assessed by intrahippocampal administration of the compound prior to MWM training which significantly reduced escape latency (36.35 ± 0.81

s). Such improvement might be attributed to the multifactorial neuroprotective effects of agmatine, including modulation of NMDA receptors, inhibition of glutamate-induced excitotoxicity and antioxidant effects as well as downregulation of neuroinflammatory markers[59]. In particular, there was a similar response to shorten escape latency with agmatine-suspended solid lipid nanoparticles (37.47 ± 0.70 s), and no significant differences compared with the above-mentioned treatment groups. This suggests that both modality forms were effective in improving learning acquisition at the training stage. Indeed, both agmatine treated groups exhibited a nominal improvement in the task compared with group receiving standard therapy (40.47 ± 0.78 s), though differences did not reach significance. Such a huge boost in cognitive domains points towards a potent effect of agmatine on cognition in this model. Collectively, these findings demonstrate that agmatine in an advanced delivery system reverts 6-OHDA-induced spatial learning deficits and restores hippocampal-based cognitive function.[57][58].

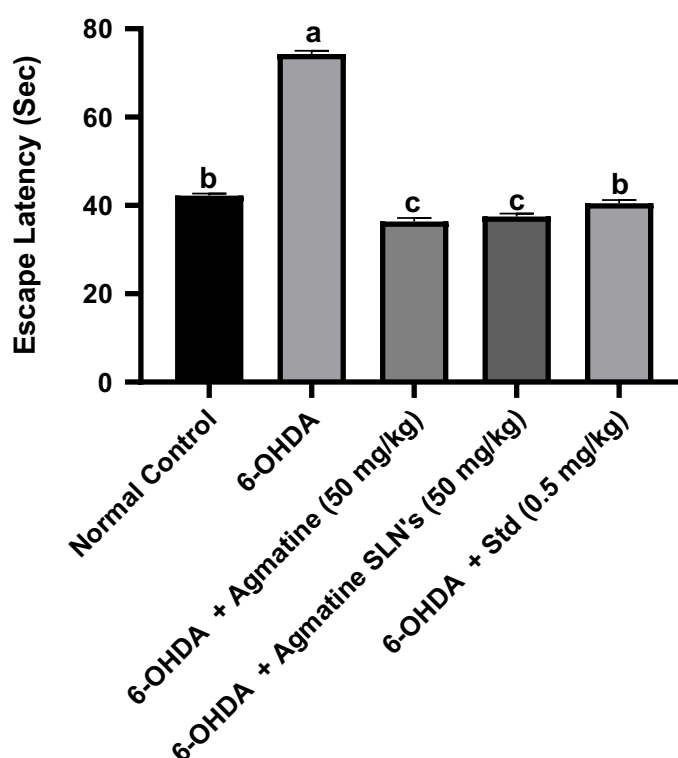


Figure. 2 Effects of Agmatine SLNs on MWM-induced impairments in spatial learning in experimental rats with neurodegeneration caused by 6-OHDA. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, and the superscripts for each parameter showed significant differences.)

Table. 3 Effect of Agmatine SLN's on spatial learning deficits by MWM on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	Escape Latency (Sec)
1.	Normal Control	42.20 ± 0.50 ^b
2.	Neurotoxicant Control (e.g., 6-OHDA)	74.33 ± 0.70 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	36.35 ± 0.81 ^c
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	37.47 ± 0.70 ^c
5.	6-OHDA + Std (0.5 mg/kg)	40.47 ± 0.78 ^b

The amount of time spent in the target quadrant after 6-OHDA was significantly reduced (26.58 ± 0.93 s) compared to the normal control (56.67 ± 0.62 s; $p < 0.05$), suggesting impaired memory recall. Agmatine SLNs outperformed the normal control group ($p < 0.05$) and showed the greatest improvement (59.33 ± 0.76 s; $p < 0.05$ vs. 6-OHDA; $p < 0.05$ vs. agmatine). Target quadrant time was significantly increased by native

agmatine (48.62 ± 0.80 s; $p < 0.05$ in comparison to 6-OHDA)[60] (Fig. 3, Table 4). Performance was greatly improved by conventional medicine (55.50 ± 0.74 s; $p < 0.05$ vs. 6-OHDA), although it was still less than that of the SLN-treated group ($p < 0.05$). The results indicate that the agmatine SLN formulation enhances memory retention and spatial recall more effectively than free agmatine and conventional therapy[61].

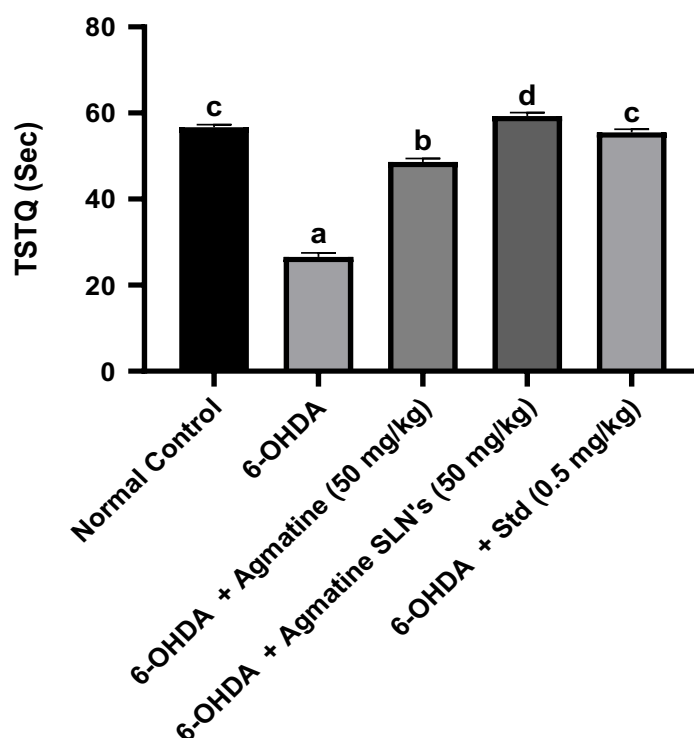


Figure. 3 Agmatine SLNs' impact on MWM-induced spatial learning deficits in experimental rats with 6-OHDA-induced neurodegeneration[62]. (All values were mean \pm SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 4 Effect of Agmatine SLN's on spatial learning deficits by MWM on 6-OHDA induced neuro degeneration in experimental rats[63].

S. No.	Groups	Time Spent in Target Quadrant (TSTQ) (Sec)
1.	Normal Control	56.67 \pm 0.62 ^c
2.	Neurotoxicant Control (e.g., 6-OHDA)	26.58 \pm 0.93 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	48.62 \pm 0.80 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	59.33 \pm 0.76 ^d
5.	6-OHDA + Std (0.5 mg/kg)	55.50 \pm 0.74 ^c

3.3. Passive Avoidance Test

Passive avoidance results indicating 6-OHDA strongly impaired memory retention and consolidation. The neurotoxicant group manifested a considerable rise in step down errors (13.03 ± 0.78) as compared to normal control (8.10 ± 0.85 ; p < 0.05), also with delay in accessing to shock free zone (43.54 ± 0.95 s vs distorted N(34-04) (Fig. 5 & 6, Table 6 & 7). These numeric changes indicate widespread disruption of associative and aversive memory processes likely mediated by the depletion of dopamine in hippocampal-amygdalar circuits underlying emotional learning [66]. Agmatine, a native treatment previously described benefits significantly inhibited the observed deficits. Latency was reduced to 12.65 ± 0.78 s (p < 0.05 vs. 6-OHDA) and the number of step-down errors was decreased to 3.63 ± 0.80 (p < 0.05 vs. 6-OHDA). Significantly, these values were lower than those of the normal control

group, which indicates a potent facilitatory effect on retention and consolidation [70]. Such a fast-acting effect would be consistent with the powerful neuromodulatory actions of agmatine, which include NMDA receptor modulation and anti-oxidative properties [64], [65]. Agmatine SLNs significantly increased both the latency (to 16.33 ± 0.88 s) and errors (4.42 ± 0.67 , p < 0.05 vs. 6-OHDA) of retention as well [67]. Nonetheless, relative to native agmatine the SLN group was trending towards higher latencies as well error counts (p < 0.05 across groups; agmatine vs. SLNs), indicating that SLNs may potentially confer a milder acute effect than native agmatine [69]. Free agmatine, however, may be more syntactically particular to acute cognition in aversive memory paradigms as these data suggest that although SLNs offer robust cognitive protection[68].

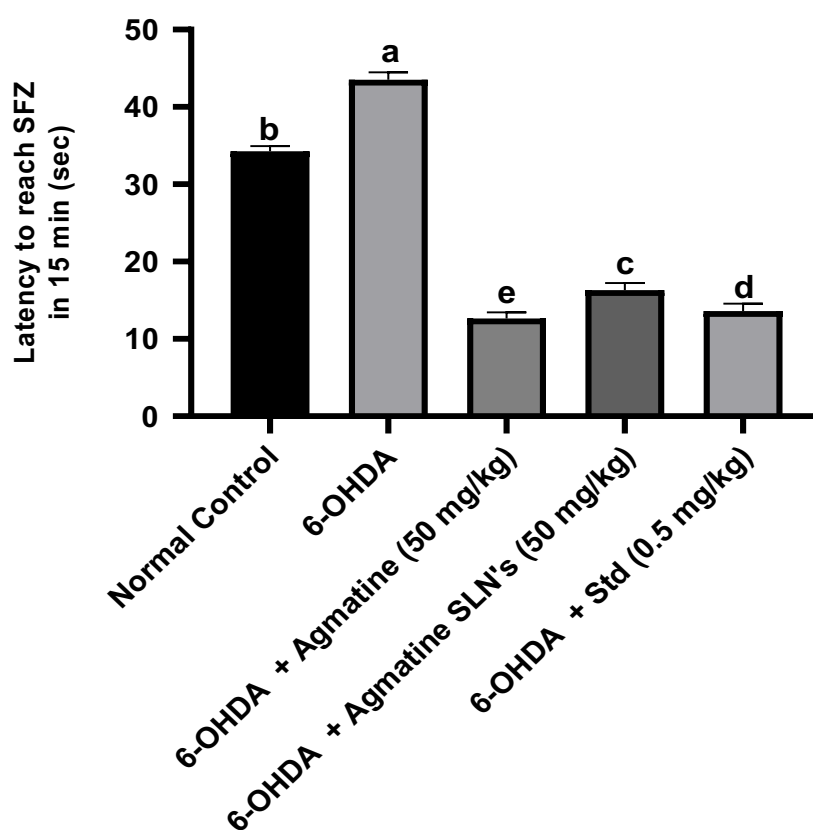


Figure. 5 Effect of Agmatine SLN's on learning and long-term memory by passive avoidance test on 6-OHDA induced neuro degeneration in experimental rats. (All values were mean \pm SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 6 Effect of Agmatine SLN's on learning and long-term memory by passive avoidance test on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	Latency to reach SFZ in 15 min (Sec)
1.	Normal Control	34.27 \pm 0.66 ^b
2.	Neurotoxicant Control (e.g., 6-OHDA)	43.54 \pm 0.95 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	12.65 \pm 0.78 ^e
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	16.33 \pm 0.88 ^c
5.	6-OHDA + Std (0.5 mg/kg)	13.60 \pm 0.96 ^d

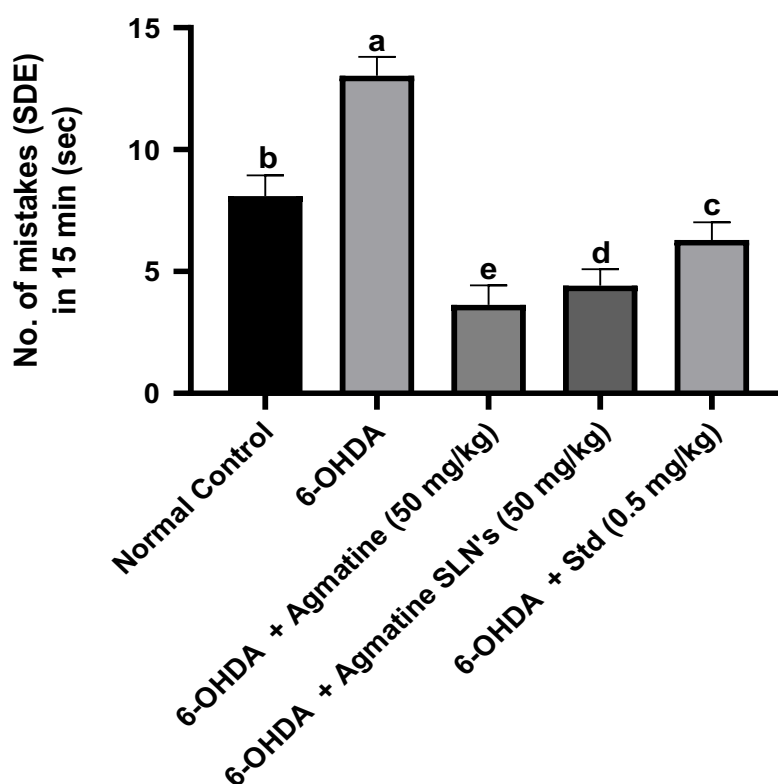


Figure. 6 Agmatine SLNs' impact on learning and long-term memory in experimental rats with 6-OHDA-induced neurodegeneration as determined by a passive avoidance test. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, and the superscripts for each parameter showed significant differences.)

Table. 7 Effect of Agmatine SLN's on learning and long-term memory by passive avoidance test on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	No. of Mistakes (SDE) in 15 min (Sec)
1.	Normal Control	8.10 ± 0.85 ^b
2.	Neurotoxicant Control (e.g., 6-OHDA)	13.03 ± 0.78 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	3.63 ± 0.80 ^e
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	4.42 ± 0.67 ^d
5.	6-OHDA + Std (0.5 mg/kg)	6.30 ± 0.72 ^c

3.4. Rotarod Test

After giving 6-OHDA, the rotarod retention time dropped from 4.32 ± 0.28 s in the control group to 2.42 ± 0.51 s ($p < 0.05$) (Fig. 7, Table 8). This shows that motor coordination got worse. The decline of dopaminergic neurons in the nigrostriatal pathway leads to a significant reduction in balance, grip strength, and neuromuscular coordination, evidenced by an approximate 44% decrease [71]. The reduced retention time signifies a disturbance in these interconnected motor circuits, as the rotarod task specifically assesses motor integration involving the cerebellum, basal ganglia, and motor cortex. Administration of native agmatine markedly enhanced motor function, resulting

in a retention time of 3.99 ± 0.49 seconds ($p < 0.05$ compared to 6-OHDA). Agmatine solid lipid nanoparticles showed a slightly better recovery rate than 6-OHDA (4.19 ± 0.38 s; $p < 0.05$). The retention time also increased to 4.16 ± 0.28 seconds with standard treatment ($p < 0.05$ compared to 6-OHDA) [72]. There was no statistically significant difference ($p > 0.05$) among SLNs (4.19 ± 0.38 s), agmatine (3.99 ± 0.49 s), and standard treatment (4.16 ± 0.28 s), indicating that they were all equally effective. The results show that all of the treatments helped with motor problems caused by 6-OHDA, but the SLN formulation did not help with this specific motor coordination test [73], [74],[75].

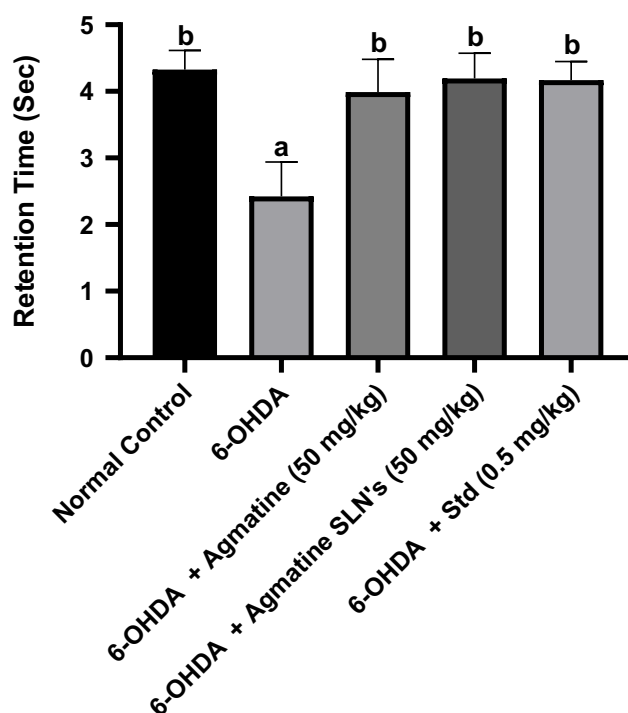


Figure. 7 Agmatine SLNs' impact on motor coordination in experimental rats with 6-OHDA-induced neurodegeneration as measured by the rotarod test. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 8 Effect of Agmatine SLN's on motor coordination by rotarod test on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	Retention Time (Sec)
1.	Normal Control	4.32 ± 0.28 ^b
2.	Neurotoxicant Control (e.g., 6-OHDA)	2.42 ± 0.51 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	3.99 ± 0.49 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	4.19 ± 0.38 ^b
5.	6-OHDA + Std (0.5 mg/kg)	4.16 ± 0.28 ^b

3.5. Locomotor Activity

By day 14, 6-OHDA administration produced marked motor impairment, as reflected by a significant reduction in locomotor activity to 185.3 ± 13.05 counts/5 min compared with 317.0 ± 22.62 counts/5 min in the normal control group (p < 0.05) (Fig 8, Table 9). This decline confirms progressive dopaminergic degeneration and reduced spontaneous motor behavior [78]. Notably, native agmatine failed to reverse this deficit, with activity remaining at 184.6 ± 22.81 counts/5 min (p > 0.05 vs. 6-OHDA), indicating limited long-term efficacy in sustaining motor function. In contrast, agmatine SLNs

significantly improved locomotor counts to 270.3 ± 22.36 counts/5 min (p < 0.05 vs. 6-OHDA), comparable to the standard medication group (286.5 ± 43.13 counts/5 min; p < 0.05) [76]. Both values were not significantly different from the normal control (p > 0.05), suggesting substantial functional recovery. The superior performance of SLNs over free agmatine highlights the importance of enhanced brain delivery and sustained release. These findings indicate that nanoencapsulation enables prolonged neuroprotection and better preservation of motor activity over time [77], [79].

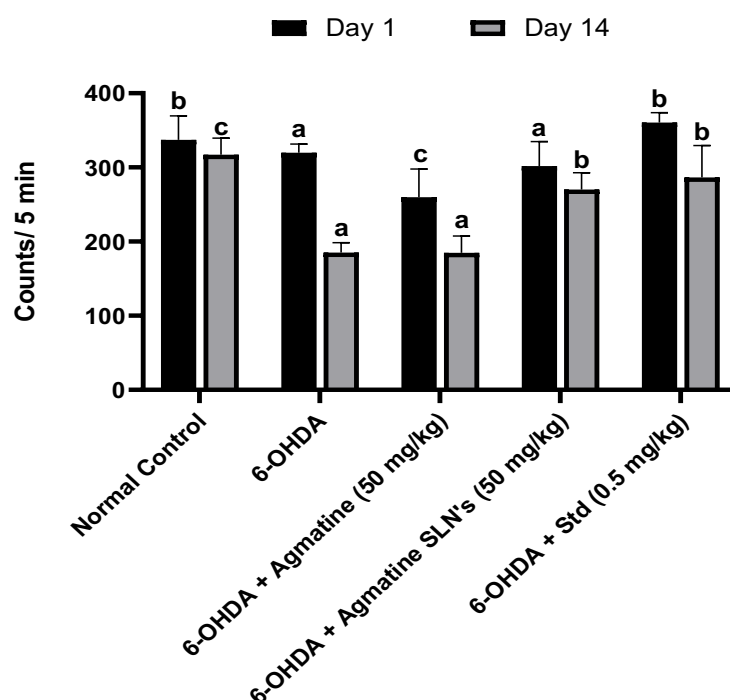


Figure. 8 Agmatine SLNs' impact on locomotor activity in experimental rats with neurodegeneration caused by 6-OHDA. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 9 Effect of Agmatine SLN's on locomotor activity on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	Locomotor Activity (Counts / 5 min)	
		Day 1	Day 14
1.	Normal Control	337 ± 32.28 ^b	317.0 ± 22.62 ^c
2.	Neurotoxicant Control (e.g., 6-OHDA)	319.6 ± 11.51 ^a	185.3 ± 13.05 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	259.6 ± 38.49 ^c	184.6 ± 22.81 ^a
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	301.6 ± 33.38 ^a	270.3 ± 22.36 ^b
5.	6-OHDA + Std (0.5 mg/kg)	360.5 ± 13.28 ^b	286.5 ± 43.13 ^b

3.6. Beam Walk Test

The 6-OHDA injection markedly reduced the duration on the beam (25.64 ± 0.32 s) and elevated the frequency of slips (9.40 ± 1.14) relative to the control group (5.75 ± 0.50 slips; 48.24 ± 0.68 s; $p < 0.05$)[80] (Fig. 9, Table 10). Treatment with native agmatine markedly enhanced performance, decreasing slip counts (5.20 ± 1.30 ; $p < 0.05$ vs. 6-OHDA) and increasing beam time (35.14 ± 0.48 s; $p < 0.05$ vs. 6-OHDA)[81]. Agmatine SLNs produced a more notable improvement in coordination after normalizing slip counts (5.00 ± 0.71 ; $p > 0.05$

compared to normal control) and a prolonged beam time (39.23 ± 0.70 s; $p < 0.05$ compared to 6-OHDA; $p < 0.05$ compared to agmatine). The slip counts (5.60 ± 0.55) were similar in both treatment groups ($p > 0.05$); however, traditional medicine exhibited the most significant recovery in beam time (44.52 ± 0.54 s; $p < 0.05$ vs. 6-OHDA)[82]. The results indicate that agmatine solid lipid nanoparticles significantly enhance balance and coordination relative to native agmatine. Nonetheless, beam-walking efficacy remains inferior to that of traditional therapy[83].

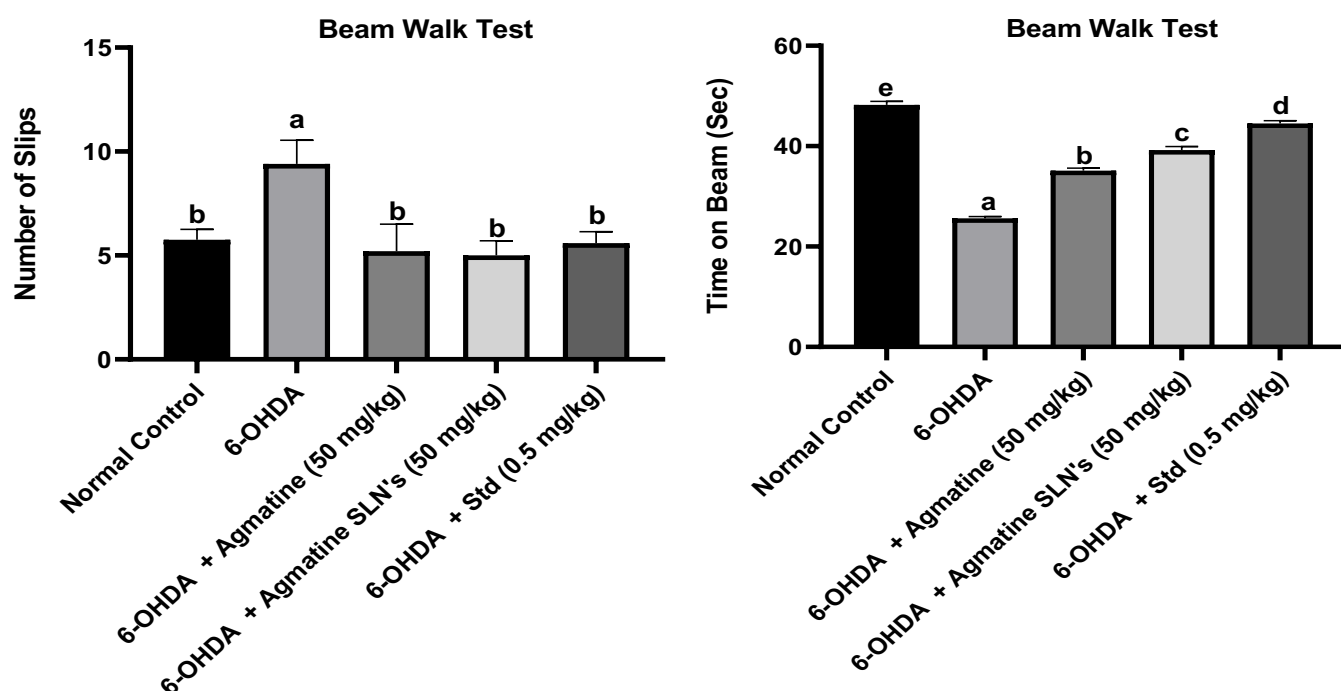


Figure. 9 Agmatine SLNs' effects on balance and coordination in experimental rats with neurodegeneration caused by 6-OHDA. A) The quantity of slips B) The amount of time on beam[84]. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 10 Effect of Agmatine SLN's on balance and coordination on 6-OHDA induced neuro degeneration in experimental rats[85]. A) Number of Slips B) Time spent on beam

S. No.	Groups	Beam Walk Test	
		No. of Slips	Time on Beam (Sec)
1.	Normal Control	5.75 ± 0.50 ^b	48.24 ± 0.68 ^e
2.	Neurotoxicant Control (e.g., 6-OHDA)	9.40 ± 1.14 ^a	25.64 ± 0.32 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	5.20 ± 1.30 ^b	35.14 ± 0.48 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	5.00 ± 0.71 ^b	39.23 ± 0.70 ^c
5.	6-OHDA + Std (0.5 mg/kg)	5.60 ± 0.55 ^b	44.52 ± 0.54 ^d

3.7. Open Field Test

In comparison to the normal control group (125.75 ± 11.63 line crossings; 9.75 ± 0.77 canter entries; p < 0.05), the neurotoxicant group showed noticeably fewer line crossings (77.50 ± 7.32) and canter zone entrances (2.00 ± 0.70) (Fig. 10, Table 11) [86]. This suggests that they were more anxious and had less mobility. Treatment with native agmatine considerably enhanced exploratory behaviour (91.40 ± 9.56 line crossings; 5.75 ± 1.99 canter entry; p 0.05 vs. 6-OHDA), even though the

recovery was only partial. In comparison to conventional medicine (83.25 ± 8.45 line crossings; 8.50 ± 1.19 canter entries; p > 0.05 vs. agmatine SLNs), agmatine SLNs showed a superior augmentation (98.25 ± 10.00 line crossings; 7.80 ± 1.77 canter entries; p 0.05 vs. 6-OHDA; p 0.05 vs. agmatine) [89]. These findings show that agmatine SLN treatment is more effective than the unencapsulated drug at reestablishing anxiety-related and locomotor behaviours[87], [88].

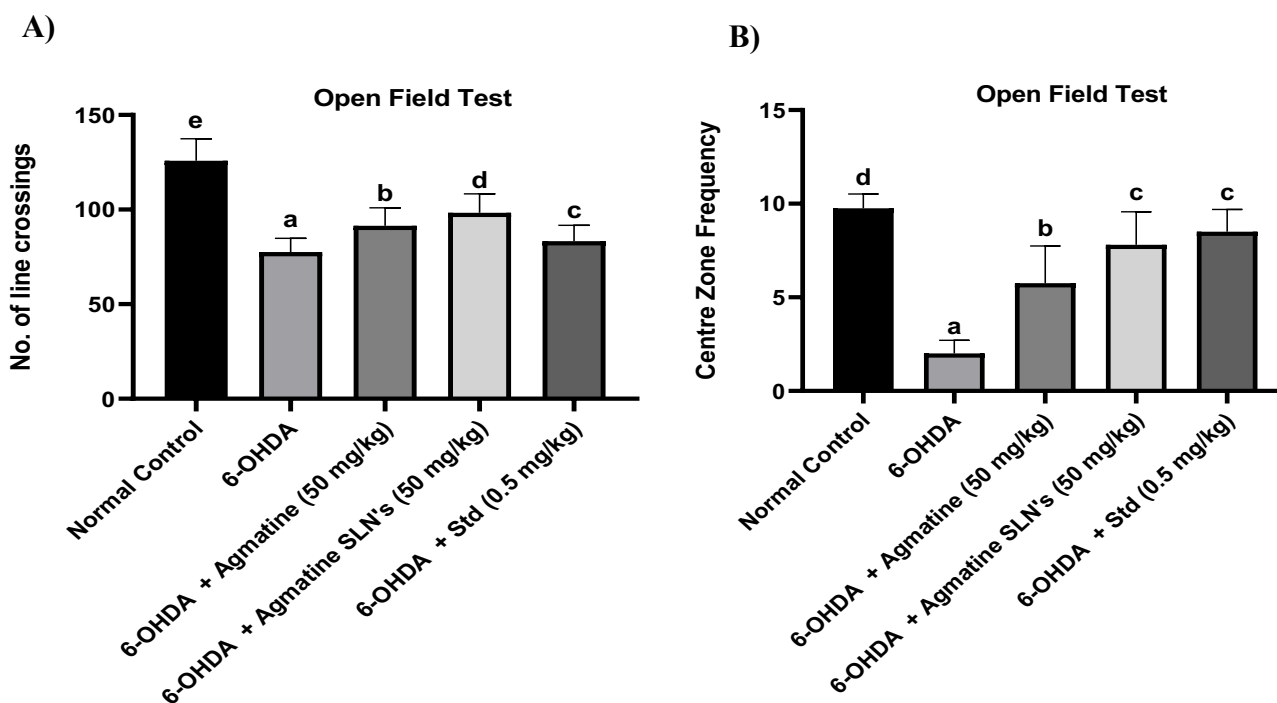


Figure. 10 Agmatine SLNs' effects on anxiety and mobility in experimental rats with 6-OHDA-induced neurodegeneration. A) The quantity of line intersections B) Frequency of the center zone. (All values were mean \pm SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 11 Effect of Agmatine SLN's on mobility and anxiety on 6-OHDA induced neuro degeneration in experimental rats. A) Number of line crossings B) Centre zone frequency

S. No.	Groups	Open Field Test	
		No. of line crossings	Centre zone Frequency
1.	Normal Control	125.75 \pm 11.63 ^c	9.75 \pm 0.77 ^d
2.	Neurotoxicant Control (e.g., 6-OHDA)	77.50 \pm 7.32 ^a	2.00 \pm 0.70 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	91.40 \pm 9.56 ^b	5.75 \pm 1.99 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	98.25 \pm 10.00 ^d	7.80 \pm 1.77 ^c
5.	6-OHDA + Std (0.5 mg/kg)	83.25 \pm 8.45 ^c	8.50 \pm 1.19 ^c

3.8. Cylinder Test

Comparing the 6-OHDA injection to the normal control, the asymmetry score was significantly lower (0.41 ± 0.023 versus 0.54 ± 0.007 ; p < 0.05) (Fig. 11, Table 12). Significant unilateral motor dysfunction is indicated by this. Motor symmetry showed partial repair after native agmatine was administered (0.50 ± 0.01 ; p < 0.05 compared to 6-OHDA), although the results were noticeably lower than those of the normal control (p <

0.05). On the other hand, the asymmetry score was returned to almost normal levels by the conventional therapy (0.52 ± 0.007 ; p > 0.05 compared to agmatine SLNs) (0.53 ± 0.005 ; p < 0.05 compared to 6-OHDA; p > 0.05 compared to normal control). The findings show that, in comparison to native agmatine, the SLN formulation provides improved functional motor recovery[90], [91].

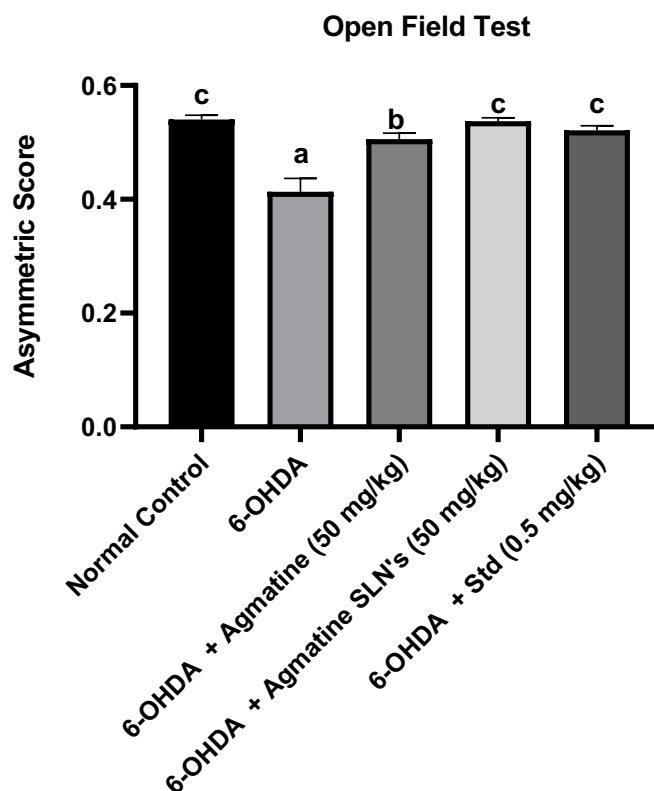


Figure. 11 Agmatine SLNs' impact on unilateral motor impairment in experimental mice with neurodegeneration brought on by 6-OHDA. (All values were mean ± SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts.)

Table. 12 Effect of Agmatine SLN's on unilateral motor dysfunction on 6-OHDA induced neuro degeneration in experimental rats.

S. No.	Groups	Asymmetric Score
1.	Normal Control	0.54 ± 0.007 ^c
2.	Neurotoxicant Control (e.g., 6-OHDA)	0.41 ± 0.023 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	0.50 ± 0.01 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	0.53 ± 0.005 ^c
5.	6-OHDA + Std (0.5 mg/kg)	0.52 ± 0.007 ^c

3.9. Light and Dark Model Test

Compared to the normal control (80.56 ± 0.56 s; 12.00 ± 0.64 transitions; 64.35 ± 0.68 s; p < 0.05), 6-OHDA significantly reduced the amount of time spent in the illuminated area (35.21 ± 0.86 s), the frequency of light–dark transitions (3.65 ± 0.15), and the latency to enter the dark compartment (20.36 ± 0.86 s) (Fig. 12, Table 13). Treatment with native agmatine considerably decreased anxiety-related measures, even though the values were still significantly different from normal controls (p < 0.05). In comparison to 6-OHDA, it increased the amount of time spent in the lit zone (46.35 ± 0.69 s; p < 0.05), the number of transitions (6.55 ± 0.36; p < 0.05), and the time it took to enter the dim zone (35.16 ± 1.69 s; p < 0.05). Agmatine SLNs, on the other

hand, had a more noticeable impact on anxiety, considerably increasing the time spent in the light area (58.12 ± 0.45 s; p < 0.05 compared to 6-OHDA and agmatine), the frequency of transitions (8.96 ± 0.25; p < 0.05 compared to 6-OHDA and agmatine), and the duration to enter the dark compartment (55.36 ± 1.23 s; p < 0.05 compared to 6-OHDA and agmatine). Agmatine SLNs had similar effects to the standard therapy (70.23 ± 0.89 s light time; 9.63 ± 0.56 transitions; 59.36 ± 0.89 s delay; p > 0.05 compared to SLNs). These results show that SLN treatment consistently outperforms native agmatine and closely resembles conventional therapy in reducing anxiety-like behavior[92].

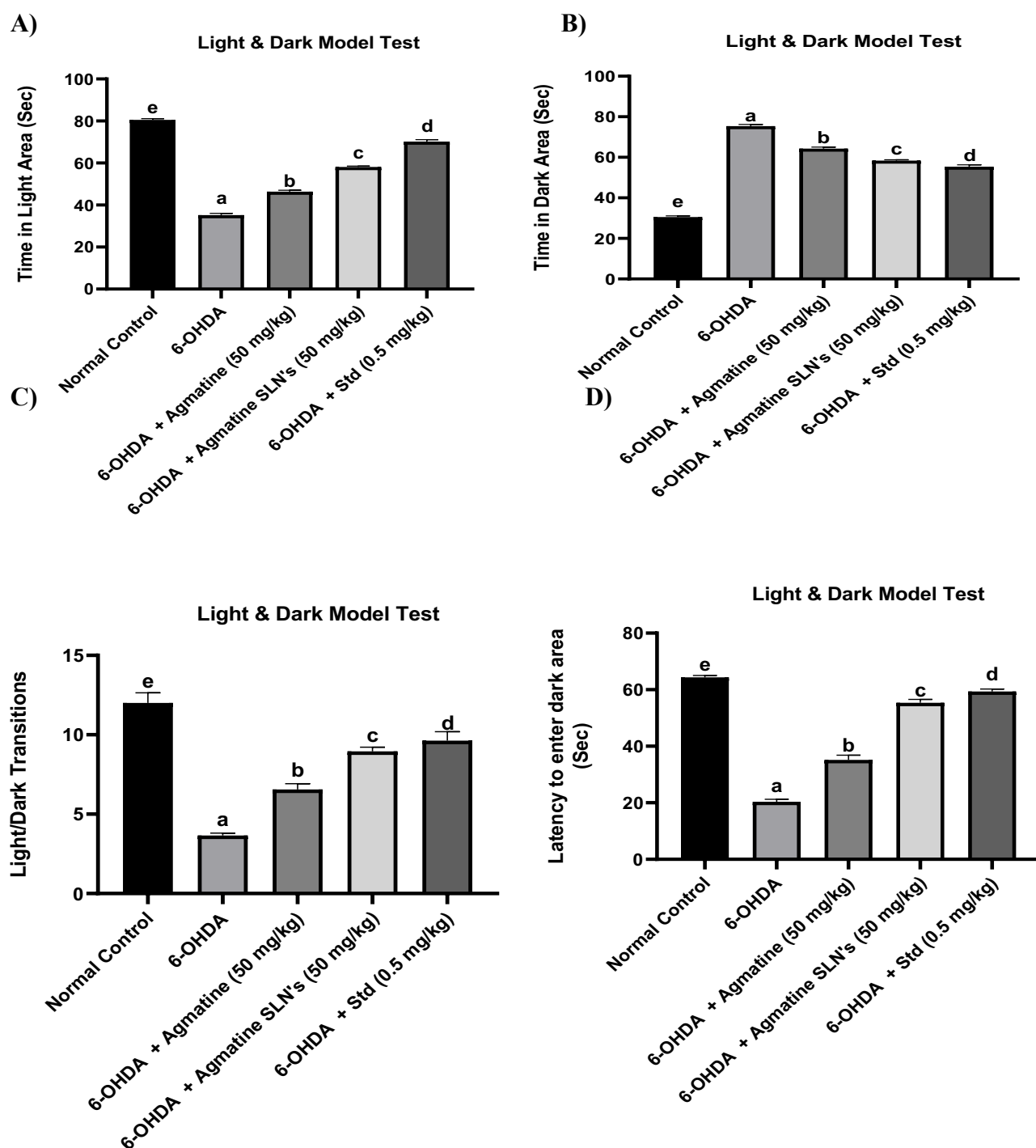


Figure. 12 Agmatine SLNs' impact on anxiety-related metrics using the light and dark model test in experimental rats with 6-OHDA-induced neurodegeneration. A) Time in Light Area (Sec), B) Time in Dark Area (Sec), C) Light or Dark Transitions D) Latency to enter dark area (Sec). (All values were mean \pm SEM, n = 3, p < 0.05 for all groups, with significant differences across each parameter's superscripts).

Table. 13 Effect of Agmatine SLN's on anxiety-related measures by light and dark model test on 6-OHDA induced neuro degeneration in experimental rats.

Groups	Light and Dark Model Test

S. No		Time in Light Area (Sec)	Time in Dark Area (Sec)	Light/Dark Transitions	Latency to enter Dark Area (Sec)
1.	Normal Control	80.56 ± 0.56 ^c	30.54 ± 0.35 ^c	12 ± 0.64 ^c	64.35 ± 0.68 ^c
2.	Neurotoxicant Control (6-OHDA)	35.21 ± 0.86 ^a	75.36 ± 0.92 ^a	3.65 ± 0.15 ^a	20.36 ± 0.86 ^a
3.	6-OHDA + Agmatine (50 mg/kg)	46.35 ± 0.69 ^b	64.32 ± 0.78 ^b	6.55 ± 0.36 ^b	35.16 ± 1.69 ^b
4.	6-OHDA + Agmatine SLN's (50 mg/kg)	58.12 ± 0.45 ^c	58.36 ± 0.56 ^c	8.96 ± 0.25 ^c	55.36 ± 1.23 ^c
5.	6-OHDA + Std (0.5 mg/kg)	70.23 ± 0.89 ^d	55.46 ± 0.46 ^d	9.63 ± 0.56 ^d	59.36 ± 0.89 ^d

4. Conclusion

In the present study, a 6-hydroxydopamine (6-OHDA)-lesioned rat model of neurodegeneration was utilized for examining the neuroprotective potential of agmatine and its SLN formulation. Data indicates that the neuroprotective effects of agmatine-loaded SLNs exceed those obtained with free agmatine and exhibit a greater degree of reproducibility across distinct behavioral paradigms, designed to assess motor and non-motor functions. The experimental model of severe cognitive impairment was validated by the intracerebroventricular administration of 6-OHDA which showed clear anxiolytic behavior and reduced locomotion and motor incoordination. IMP: significant with impaired transfer latency of EPM, escape latency as well as target quadrant time in MWM; low counts of locomotion, abnormalities of beam walking, scores for cylinder asymmetry and parameters for light-dark box (LDB) also were prominent as passive avoidance retention. Collectively, these modifications recapitulate the complex neurodegenerative pathophysiology with concomitant vasodegeneration and dopaminergic as well as non-dopaminergic aspects.

Agmatine SLN treatment effectively reversed the cognitive deficits evinced by a decreased transfer latency in the Elevated plus Maze and remarkable enhancements in spatial learning and memory retention noted with a Morris Water Maze. IMPORTANCE: The SLN formulation significantly enhanced time in target quadrant compared with free agmatine, and sometimes was similar or superior to standard therapy. Notably, Agmatine SLNs show comparable efficacy to standard treatment and retained superiority over native agmatine in anxiety-related behavior models (Open Field test and Light-Dark model). This suggests they have a greater potential for modifying non-motor symptoms. Agmatine SLNs showed a significant improvement in balance and coordination (locomotor activity, beam walk), forelimb symmetry (cylinder test) and rotarod performance. SLN formulation significantly sustained motor performance during follow-up period compared to agmatine free ($p < 0.01$) at proximity of normal values and proved to be more bioavailable and prolonged availability in central nervous system.

In conclusion, our results demonstrate that SLN-mediated delivery significantly improves agmatine activity in preclinical models of neurodegeneration. Since also our data indicate that this novel nanotechnological strategy for targeted delivery of agmatine to brain, develop a potential disease-modifying therapy in the progressive neurodegenerative diseases characterized by these motor and non-motor deficits. Further studies will be required to determine precisely how this formulation operates and could potentially be integrated into the clinic.

Declaration of conflict of interest

The authors declare no conflict of interest.

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