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# Neuroprotective Potential of Agmatine in Mitigating Aluminium Chloride-Induced Cognitive Deficits in Rats

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**Abstract:**

**Introduction:** Alzheimer's disease (AD), a progressive neurodegenerative disorder, is characterized by cognitive decline, memory loss, and impaired executive function. $AlCl_3$ is known to exacerbate oxidative stress and neuroinflammation, contributing to cognitive deficits similar to those observed in AD. Agmatine, a polyamine derived from arginine, has emerged as a potential neuroprotective agent due to its antioxidant, anti-inflammatory, and neuromodulatory properties.

**Aim & Objectives:** This study aims to evaluate the neuroprotective effects of agmatine against aluminium chloride ($AlCl_3$)-induced cognitive impairments in male Wistar rats. The objectives include assessing behavioural changes through locomotor activity, the Morris Water Maze, and Object Recognition Task, along with biochemical and neurochemical alterations, focusing on oxidative stress and hippocampal glutamate levels as potential mechanisms underlying cognitive deficits.

**Material & Methods:** A total of thirty male Wistar rats (n = 6) were split up into five groups. For 21 days, groups II, III, IV, and V were treated with $AlCl_3$ (100 mg/kg, p.o.) and agmatine at doses of 3 mg/kg (i.p.), 10 mg/kg (i.p.), and 30 mg/kg (i.p.), respectively, while group I (control) was given 0.9% saline. The Morris Water Maze (MWM) and Object Recognition Task (ORT) were used to evaluate cognitive abilities. In brain tissues, biochemical and neurochemical studies were examined.

**Result:** Prolonged exposure to $AlCl_3$ raised acetylcholinesterase activity, nitrite, MDA, and decreased GSH levels in the brain, all of which markedly impacted cognitive function. $AlCl_3$ therapy also resulted in significant decreases in the hippocampal levels of DA, NE, serotonin, GABA, and glutamate.

**Conclusion:** Agmatine modulates hippocampus glutamate levels, restores neurotransmitter levels, and reduces oxidative stress to show neuroprotective effects against $AlCl_3$-induced cognitive deficits. These results imply that agmatine has therapeutic potential in reducing neurotoxicity and cognitive impairment associated with aluminium.

**Keywords:** Alzheimer Disease, Aluminum Chloride, Agmatine.

## 1. Introduction

Memory plays a pivotal role in shaping an individual's daily life and activities. It is integral to processes such as decision-making, interpersonal relationships, and self-care. Cognition encompasses the mental processes underlying human experiences, thought, and understanding. Mild cognitive impairment (MCI) is characterized by a quantifiable decline in cognitive functions, including memory and executive abilities. Individuals diagnosed with MCI have an elevated risk of progression to Alzheimer's disease (AD) or other forms of dementia. Aluminium, a well-established neurotoxic agent, has been implicated in the pathogenesis of Alzheimer's disease. This association is attributed to its ability to cross biological barriers and accumulate within the central nervous system, thereby contributing to neurodegenerative processes (Sun et al., 2009). Aluminium compounds are commonly incorporated into a wide range of commercially produced products intended for human consumption. Aluminium sulfate (alum) is frequently used in the treatment of water intended for bottling and in municipal drinking water supplies as a clarifying agent. Additionally, aluminium is present in certain food preservatives, cookware, drinking water, and naturally occurring dietary sources (Schafer and Seifert, 2006). Furthermore, aluminium exposure has been observed to induce clinical signs and pathological features in experimental animal models that closely resemble those associated with Alzheimer's disease (AD) (Sreekumaran and Ramakrishna, 2002). Consequently, aluminium exposure has been proposed as a contributing factor in the development of cognitive decline (Shuchang et al., 2008). Moreover, an expanding body of evidence suggests that aluminium exerts neurotoxic effects through the induction of oxidative stress (Kumar et al., 2009, Newairy et al., 2009, Dua et al., 2010, Sethi et al., 2008). The administration of aluminium to rats has been shown to induce cognitive impairments and oxidative damage within the cortex and hippocampus of the brain (Thirunavukkarasu et al., 2012).

Aluminium-induced neurotoxicity has also been implicated in the pathogenesis of Alzheimer's disease (AD), an age-associated neurodegenerative disorder. AD is primarily characterized by progressive cognitive decline, accompanied by personality changes, impaired learning abilities, deterioration of motor functions, and eventual loss of language skills (Yankner, 1996). The pathological hallmarks of aluminium-induced Alzheimer's disease (AD) include the presence of senile plaques, which are spherical aggregations of β-amyloid protein, accompanied by degenerating neuronal processes. Additionally, neurofibrillary tangles, consisting of paired helical filaments and other associated proteins, are also observed.

Aluminium toxicity and its associated impact on Alzheimer's disease (AD) primarily affect individuals in middle to older age, with the prevalence of the condition showing a significant increase after the age of 65 (Butterfield et al., 2013). Among the familial and sporadic forms of Alzheimer's disease (AD), sporadic AD accounts for approximately 95% of cases. It arises from a complex interplay of various etiological factors (Barone et al., 2014). Numerous postmortem studies of brains from Alzheimer's disease (AD) patients suggest that mitochondrial dysfunction and the accumulation of amyloid β (Aβ) at synapses may contribute to synaptic damage, impair neurotransmission, and drive cognitive decline in both elderly individuals and AD patients. Despite ongoing research, the development of an effective treatment for AD remains elusive (Bonda et al., 2010). Several studies have reported that aluminium toxicity can lead to the formation of free

radicals, oxidative stress, mitochondrial dysfunction, and inflammatory processes. These factors may interact and exacerbate each other in a vicious cycle of toxicity, ultimately resulting in neuronal impairment, cellular dysfunction, and, eventually, cell death (Butterfield et al., 2013).

Agmatine, a cationic polyamine, was first identified by the German biochemist Albrecht Kossel in herring sperm during the early 20th century. It is synthesized through the decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC) (Tabor and Tabor, 1984). As an endogenous neuromodulator, agmatine has emerged as a potential therapeutic agent for the management of various central nervous system (CNS) disorders. Multiple studies have demonstrated that agmatine is synthesized in both the brain and spinal cord. Recently, agmatine has garnered significant attention due to its neuromodulatory and neuroprotective properties, leading to a growing number of investigations into its effects on the central nervous system (CNS) using various cellular and animal models. Preclinical studies, for example, have reported that agmatine exerts beneficial effects on nociception (Fairbanks et al., 2000, Santos et al., 2005), morphine tolerance (Kolesnikov et al., 1996), drug withdrawal (Uzbay et al., 2000), seizures (Bence et al., 2003, Su et al., 2004), depression (Li et al., 2003), anxiety (Moretti et al., 2014), memory (Arteni et al., 2002) and Parkinson's disease (Gilad et al., 2005). Experimental studies investigating memory and learning in aged animal models treated with agmatine have predominantly demonstrated that agmatine alleviates cognitive deficits (Song et al., 2014).

There are no studies till date that have examined the biochemical and molecular role of agmatine in counteracting neurotoxicity induced by aluminium chloride ($AlCl_3$). This research aims to address this gap by evaluating the behavioral, biochemical, and neurochemical alterations caused by $AlCl_3$ in the rat brain. Considering these factors, the present study has been designed to investigate the pharmacological potential of agmatine in mitigating aluminium chloride-induced neurotoxicity and associated cognitive decline in rats.

## 2. Materials and Methods

### 2.1 Animals

In the present study, experiments were conducted using male Wistar rats weighing 200–250 g, procured from LLRUVAS, Hisar. The animals were housed in the animal facility of ISF College of Pharmacy, Moga, Punjab, India, and provided with food and water ad libitum. The rats were maintained in polyacrylic cages under standard husbandry conditions, including a controlled room temperature of 22±2°C, relative humidity of 55–60%, and a 12-hour light/dark cycle (lights on at 7:00 AM). Behavioral analyses were performed during the animals' active phase, between 19:00 and 23:00 hours. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with the guidelines of the Indian National Science Academy (INSA) for the care and use of experimental animals.

### 2.2 Drugs and chemicals

Aluminium chloride ($AlCl_3$) was purchased from LOBA, INDIA, whereas agmatine was purchased from Sigma- Aldrich, USA. $AlCl_3$ was dissolved in saline (Abdel-Zaher et al., 2017) and Agmatine

was dissolved in distilled water. All chemicals utilized in the study were of analytical grade, and solutions of the drugs and chemicals were freshly prepared prior to use.

### 2.3 Experimental Protocol

Thirty male Wistar rats of weight 200–250 g were selected for this study. Animals were divided into five groups; each group has 6 animals.

Group 1 Vehicle control group (0.9% saline treated)

Group 2 $AlCl_3$ (100 mg/kg, p.o)

Group 3 $AlCl_{3+}$ Agmatine (3 mg/kg, i.p)

Group 4 $AlCl_{3+}$ Agmatine (10 mg/kg, i.p)

Group 5 $AlCl_{3+}$ Agmatine (30 mg/kg, i.p)

### 2.4 Experimental procedure

All experimental groups were administered aluminium chloride ($AlCl_3$) once daily for 21 consecutive days. On the 14th day, the animals received agmatine treatment at doses of 3, 10, and 30 mg/kg via intraperitoneal (i.p.) injection. Body weight measurements and behavioral observations were recorded both prior to and following the completion of the experiment. From days 15 to 19, the animals underwent the Morris water maze test as a behavioral assessment. On days 20 and 21, the animals were subjected to the Object Recognition Task (ORT) and the Open Field Test. Twenty-four hours after the final dose administration, the animals were euthanized, and their organs were harvested, cleaned, washed with phosphate-buffered saline (PBS, pH 7.4), and subsequently used for various analyses.

### 2.5 Behavioral assessment

#### 2.5.1 Open Field

On day 21, each animal was assessed for locomotor activity using the Open Field Test. The test measures both horizontal and vertical activity, as well as activity patterns. The Open Field apparatus consisted of a wooden, rectangular chamber (100×100×40 cm) with a light brown color. The floor of the apparatus was subdivided into 25 rectangular squares using pencil lines. The experimental room was illuminated by a 40-watt white bulb positioned 150 cm above the apparatus. The animal was placed at the center of the apparatus two hours after an initial exposure. During the 10-minute observation period, the number of squares crossed, grooming behavior, and rearing events were recorded. A crossing was defined as the animal placing all four paws into a new square. After each trial, the apparatus was thoroughly cleaned. Total locomotor activity was calculated by summing the number of squares crossed, grooming events, and rearing occurrences over the 10-minute period (Thangarajan et al., 2014).

#### 2.5.2 Object recognition test (ORT)

The Object Recognition Task (ORT) was conducted with minor modifications to the standard procedure (Pitsikas et al., 2001, Giorgetti et al., 2010). The test used a wooden open box (80 × 60 × 40 $cm^3$) with two distinct wooden objects (triangle and cylinder, ~10 cm height) for discrimination.

Rats habituated for 15 minutes before a 3-minute sample trial (T1) where two identical objects were placed in opposite corners. After a 60-minute interval, a choice trial (T2) replaced one familiar object with a novel one, with positions counterbalanced to avoid bias. Exploration time near objects was recorded, and data analysed as total exploration in T1 and time spent on the novel versus familiar object in T2.

### 2.5.3 Morris water maze test

The Morris Water Maze is commonly employed to assess spatial memory and learning (Morris, 1984). The experiment used a circular water tank (180 cm diameter, 60 cm height) filled to a depth of 40 cm with opaque water at 25±1°C. A hidden platform (10 cm diameter) was placed 2 cm below the surface in the center of a designated quadrant. Rats underwent training over four days (days 15–18) with four trials per day, starting from different quadrants, and latency to locate the platform was recorded. On day 19, a probe test assessed memory by measuring the time spent in the target quadrant after the platform was removed.

## 2.6 Estimation of Biochemical Parameters

Terminally, on 22 day, the hippocampal rat brain regions were dissected out, homogenized and the supernatant was used for further analysis of biochemical parameters.

### 2.6.1 Brain homogenate preparation

The animals were euthanized by cervical dislocation, and their brains were carefully extracted and rinsed with ice-cold isotonic saline. Cortical and hippocampal tissue samples were then homogenized in ice-cold 0.1 mmol/L phosphate buffer (pH 7.4) at a 10% (w/v) tissue-to-buffer ratio. The resulting homogenate was subsequently centrifuged at 10,000 rpm for 15 minutes at -4°C. The supernatant obtained was collected and utilized for biochemical analyses (Kumar et al., 2011).

### 2.6.2 Protein determination

Protein was measured in all brain samples by the method of (Lowry et al., 1951) using bovine serum albumin (BSA) (1 mg/ml) as a standard.

### 2.6.3 Acetylcholinesterase activity

The quantitative estimation of acetylcholinesterase activity in brain was performed according to the method described by (Ellman et al., 1961). The assay combination contained 0.10 ml of DTNB (Ellman reagent) and 0.10 ml of acetylthiocholine iodide, 3 ml of 0.01 M sodium phosphate buffer (pH 8) and 0.05 ml of supernatant. The change in absorbance was measured instantly at 412 nm spectrophotometrically. Moreover, the acetylcholinesterase activity in the supernatant was expressed as nmol per mg protein.

### 2.6.4 Malondialdehyde (MDA)

The significant estimation of malondialdehyde (MDA) – end product of lipid peroxidation (LPO) – in brain homogenate was performed according to the method of (Wills, 1966). The amount of MDA was estimated after its reaction with thiobarbituric acid at 532 nm using spectrophotometer

(Shimadzu, UV-1700). The concentration of MDA was calculated from a standard curve and expressed as nmol per mg protein.

### 2.6.5 Reduced glutathione (GSH)

Reduced glutathione in brain was measured according to the method described by Ellman (1959). 1ml supernatant was precipitated with1ml of 4% sulfosalicylic acid and cold digested at 4°C for 1h. The samples were centrifuged at 1200g (15min). To 1 ml of the supernatant, 0.2mlof 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 2.7 ml of phosphate buffer (0.1M, pH8) were added. Moreover, the yellow colour that appears was measured instantly at 412 nm using a spectrophotometer. Moreover, the concentration of glutathione in the supernatant was determined from a standard curve and expressed as µmol per mg protein.

### 2.6.6 Nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Greiss reagent (0.1%N-[1-naphthyl] ethylenediaminedihydrochloride,1%sulfanilamide and 2.5% phosphoric acid) as described by (Green et al., 1982). The equal volumes of Greiss reagent and supernatant were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance find out at 540 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as µmol per mg protein.

### 2.6.7 Statistical analysis

The values are expressed as mean ±S.D. Moreover, the acquisition trial results (MWM) were examined by using two-way ANOVA (Analysis of variance) and followed by Bonferroni's post hoc test for many comparisons. In ORT, comparisons of total time of exploration during T1 and T2 on familiar and novel object were analysed by paired t-test. The discriminative index, results of probe trial in MWM model and biochemical and parameters were analysed using one way ANOVA followed by Tukey's test, using statistical Graph Pad Prism software (version5.0, La Jolla, CA, USA). The $P < 0.05$ was set to be statistically significant.

## 3. Results:

### 3.1 Behavioral Parameters

### 3.1.1 Effect of agmatine on spontaneous locomotor activity in $AlCl_3$ treated rats.

In open field test, $AlCl_3$ treated rats showed significantly decreased locomotor activity as compared with vehicle control group ($P<0.05$). But their performance was significantly improved by chronic treatment with agmatine ($p<0.05$). Agmatine at 10 and 30mg/kg dose was found to be effective in ameliorating $AlCl_3$ induced decreased locomotor activity and restored toward normal on $21^{st}$ day ($p<0.05$) (Fig. 1).

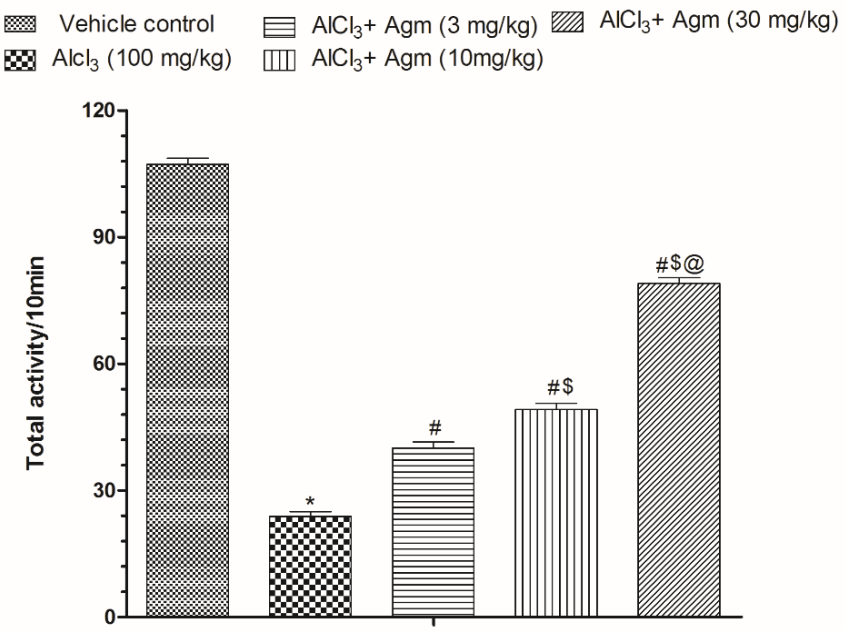


**Figure.1 Effect of agmatine on spontaneous locomotor activity in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg). Agm-Agmatine

### 3.1.2 Effect of agmatine on memory performance in Morris water maze (MWM) task in $AlCl_3$ treated rats.

In the Morris water maze task, $AlCl_3$ treated rats showed poor learning abilities during acquisition trial. On day 15, there was no significant difference between the mean latencies of all groups. But the mean latencies were found to be significantly prolonged on day 16, 17 & 18 ($P<0.05$) in the $AlCl_3$ treated rats as compared with that of vehicle control, indicating learning impairment. But their performance was significantly improved by chronic treatment with agmatine ($p<0.05$). Agmatine at 10 and 30 mg/kg dose was found to be effective in ameliorating $AlCl_3$ induced spatial memory deficit.

During the probe trial on day 19, with the plateform removed, $AlCl_3$ treated rats failed to remember the precise location of the plateform, spending significantly less time in the target quadrant than vehicle treated group [$p<0.05$, Fig. 13 B]. But the agmatine treated $AlCl_3$ rats spend significantly more time in target quadrant ($p<0.05$) as compared with $AlCl_3$ alone indicating improved consolidation of memory. Moreover Agmatine (30 mg/kg) treated rats spent significantly more time in the target quadrant as compared with agmatine (3 and 10 mg/kg) (Fig. 2a and 2b).

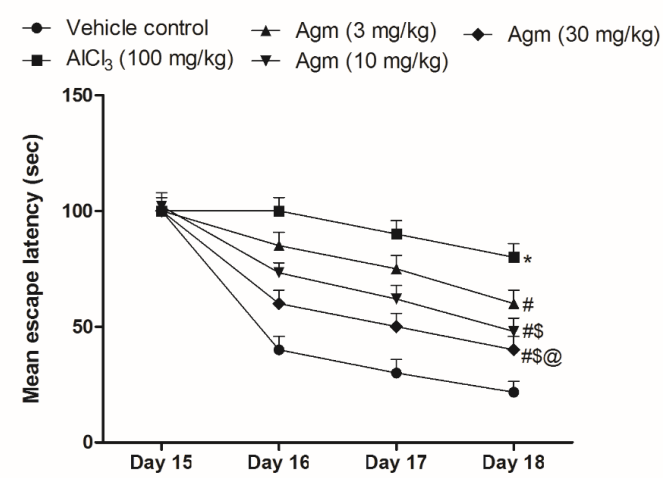


**Figure.2a Effect of agmatine on memeory performance in Morris water maze (MWM) task in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg)

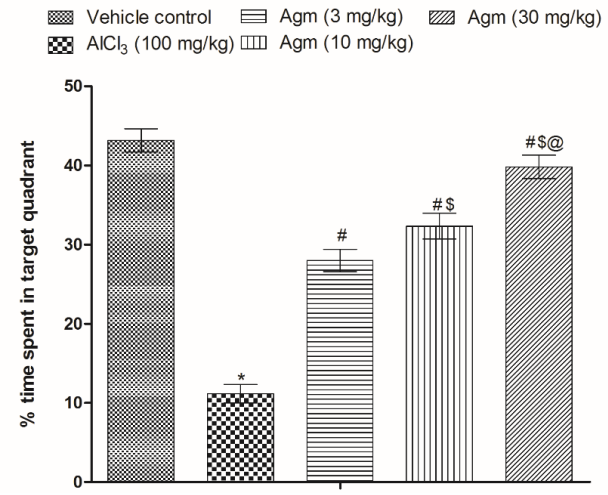


**Figure.2b Effect of agmatine on time spent in target quadrant (s) in Morris water maze (MWM) task in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg)

### 3.1.3 Effect of agmatine on memory performance in object recognition task in $AlCl_3$ treated rats.

On day 20 following $AlCl_3$ administration, in the sample phase (T1) wherein both the objects were similar, all the rats took similar time for object exploration (Fig. 12A), regardless of the treatment. Whereas, on day 21 when animals exposed with familiar and novel objects showed significant discrimination. However, $AlCl_3$ treated rats were unable to discriminate between familiar and novel

objects and spend almost equal time to explore the familiar and novel objects. Whereas, agmatine treatment produced significant improvement in discriminating ability between familiar and novel object in $AlCl_3$ treated rats and spend more time to explore novel object. Unpaired t-test comparisons showed significant difference in exploration time (Fig. 12B) and the agmatine treated rats spent more time in exploring the novel objects ($P$<0.05) (Fig. 3a and 3b).

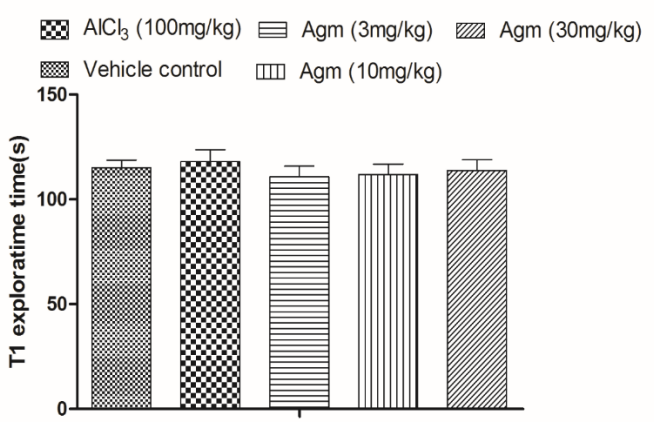


**Figure.3a Effect of Agmatine on memory performance in object recognition task in $AlCl_3$ treated rats.**

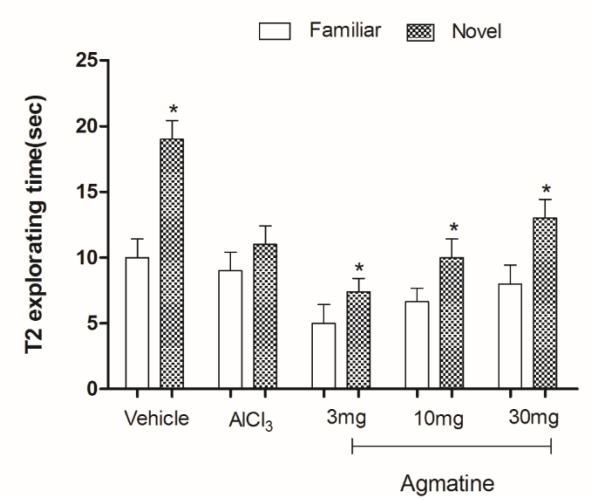


**Figure.3b Effect of agmatine on time spent exploring the familiar and novel objects during T2 in AlCl3 treated rats. *$P$<0.05 vs Familiar object**

### 3.2 Neurochemical estimation

#### 3.2.1 Effect of Agmatine on neurotransmitters (Serotonin (5-HT), Dopamine (DA) and Norepinephrine (NE) levels in $AlCl_3$ treated rats.

$AlCl_3$ treated rats showed significant decrease in the level of neurotransmitter serotonin (p<0.05), dopamine (p<0.05) and norepinephrine (p<0.05) as compared with vehicle control. However, treatment with agmatine (3, 10 and 30 mg/kg) dose dependently significantly attenuated the reduction in serotonin (p<0.05), dopamine (p<0.05) and norepinephrine (p<0.05) levels as compare

with $AlCl_3$ treated rats. Among selected doses, agmatine (10 & 30 mg/kg) was found to more effective in restoring the level of catecholamines (5-HT, DA and NE) (Fig.4).

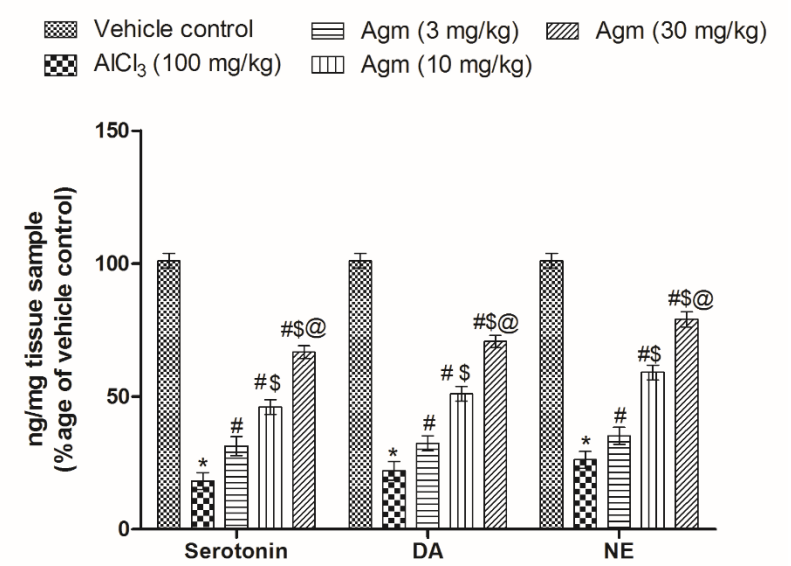


**Figure.4 Effect of agmatine on catecholamine level in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. Data analyzed by one way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg)

### 3.2.2 Effect of Agmatine on Glutamate level in $AlCl_3$ treated rats.

$AlCl_3$ treated rats showed significant increase in the level of glutamate ($p<0.05$) as compared to vehicle control. However, chronic treatment with agmatine (3, 10 and 30 mg/kg) dose dependently showed significant decrease in glutamate ($p<0.05$) level as compared with $AlCl_3$ treated rats. Among selected doses, agmatine (10 & 30mg/kg) showed more effective in prevention of alteration of glutamate levels (Fig.5).

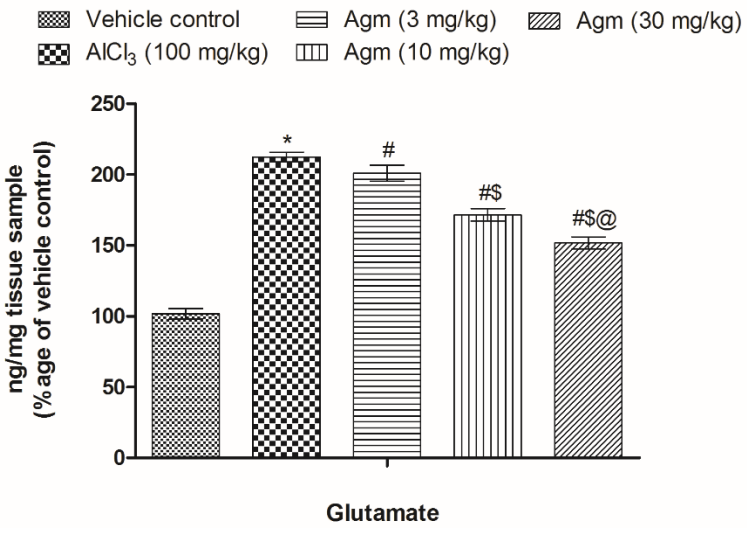


**Figure.5 Effect of Agmatine on Glutamate level in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. Data analyzed by one way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg)

### 3.2.3 Effect of Agmatine on GABA level in $AlCl_3$ treated rats.

$AlCl_3$ treated rats showed significant decrease in the level of GABA ($p<0.05$) as compared to vehicle control. However, chronic treatment with agmatine (3, 10 and 30 mg/kg) dose dependently showed significant increase in GABA ($p<0.05$) level as compared with $AlCl_3$ treated rats. Among selected doses, agmatine (10 & 30mg/kg) showed more effective in prevention of alteration of GABA levels (Fig. 6).

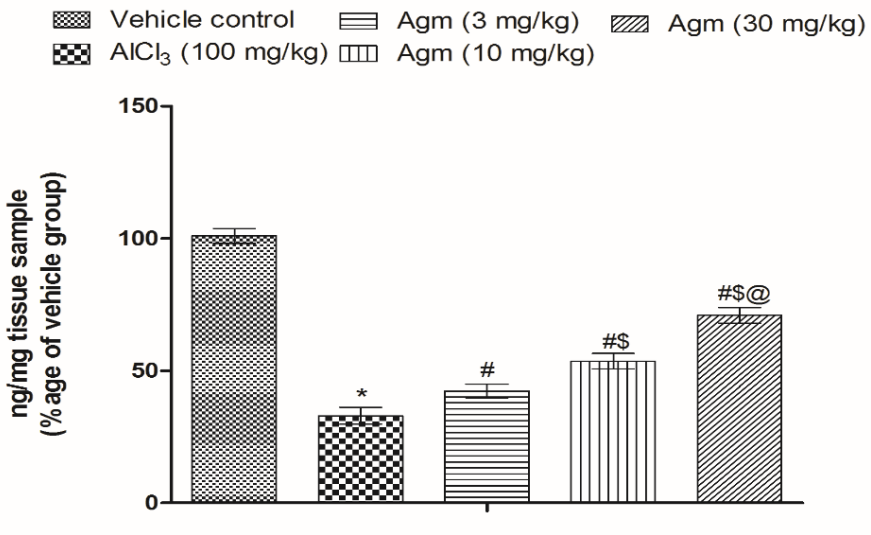


**Figure.6 Effect of Agmatine on GABA level in $AlCl_3$ treated rats.**

Values are expressed as mean ± SD. Data analyzed by one way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg)

## 3.3 Biochemical parameters

### 3.3.1 Effect of Agmatine treatment on oxidative stress parameters (MDA, GSH, Nitrite and AChE levels) in $AlCl_3$ treated rats.

$AlCl_3$ administration in rats caused elevation in malondialdehyde, nitrite, AChE level and depletion of glutathione levels ($P<0.05$) in the hippocampal regions of rat brain, indicating elevated oxidative stress. However, administration of agmatine dose dependently attenuated

$AlCl_3$ - induced elevation in oxidative stress ($P<0.05$), AChE level and restored depleted glutathione levels, suggesting its antioxidant potential (Table 1).

**Table.1 Effect of Agmatine on brain oxidative stress parameters and acetylcholinesterase activity in $AlCl_3$ treated rats.**

| Treatment | MDA (nmol/mg protein) (Lowry et al., 1951) | GSH (µ Mol/mg protein) | Nitrite (nmol/mg protein) | AChE (µ mol/min/mg protein) |
|---|---|---|---|---|
| Vehicle control | 0.092± 0.0072 | 0.098± 0.0035 | 1.88± 0.374 | 145.4± 21.79 |
| $AlCl_3$ | 0.206± 0.0106* | 0.054± 0.0011* | 3.87± 0.137* | 371.2± 53.41* |
| Agmatine (3 mg/kg) | 0.192± 0.0108$^{\#}$ | 0.062±0.0039$^{\#}$ | 2.78±0.189$^{\#}$ | 355.0± 63.91$^{\#}$ |
| Agmatine (10 mg/kg) | 0.183± 0.0115$^{\#\,\$}$ | 0.085± 0.0044$^{\#\,\$}$ | 1.97± 0.435$^{\#\,\$}$ | 321.6±62.91$^{\#\,\$}$ |
| Agmatine (30 mg/kg) | 0.144± 0.0119$^{\#\,\$\,@}$ | 0.093± 0.0045$^{\#\,\$\,@}$ | 1.96± 0.249$^{\#\,\$\,@}$ | 228.4± 39.38$^{\#\,\$\,@}$ |

Values are expressed as mean ± SD. Data analyzed by one way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ vs vehicle control, $^{\#}p<0.05$ vs $AlCl_3$, $^{\$}p<0.05$ vs $AlCl_3$+ Agmatine (3 mg/kg), @ $p<0.05$ vs $AlCl_3$+ Agmatine (10 mg/kg). MDA-Malondialdehyde, GSH- Reduced glutathione, AChE- Acetylcholinesterase.

**4. Discussion:**

The present study demonstrates the neuroprotective potential of agmatine against aluminium chloride ($AlCl_3$)-induced cognitive decline in rats. Oral administration of $AlCl_3$ in rats resulted in cognitive impairment, increased oxidative stress, neuroinflammation, and disruption of hippocampal neurotransmitter levels. Aggregates of amyloid beta (Aβ) are well-established in their ability to activate multiple neurotoxic pathways, thereby promoting neuronal cell death in various brain regions (Albrekkan and Kelly-Worden, 2013). The results of the present study are consistent with previous findings that report similar neurotoxic alterations following aluminium chloride ($AlCl_3$) administration in rats (Lakshmi et al., 2015).

n the present study, aluminium chloride ($AlCl_3$) administration induced both spatial and non-spatial memory deficits in rats. The rats exhibited impaired learning and memory in the Morris Water Maze, demonstrated an inability to distinguish between familiar and novel objects in the Object Recognition Task, and showed altered locomotor activity in the Open Field Test. These findings are in line with prior research that has also reported spatial and non-spatial learning impairments following $AlCl_3$ administration (Nampoothiri et al., 2015). The Morris Water Maze (MWM) test was originally developed to assess hippocampus-dependent cognitive functions (Morris, 1984), however, it also involves the coordinated activity of various brain regions, including the hippocampus. Whereas Recognition memory refers to the ability to distinguish between novel and familiar stimuli, and the functional integrity of the temporal lobe, particularly the hippocampus, is critical for the encoding,

storage, and retrieval of this form of memory (Pitsikas et al., 2001). Thus, In the present study, aluminium chloride ($AlCl_3$) administration appears to induce functional damage to hippocampal neurons, leading to impairments in learning and memory. It has been proposed that the cognitive deficits observed following $AlCl_3$ exposure are linked to disruptions in synaptic transmission and neurodegeneration within the hippocampal region (Abdel-Zaher et al., 2017). In this study, treatment with agmatine resulted in a significant, dose-dependent attenuation of aluminium chloride ($AlCl_3$)-induced impairments in both spatial and non-spatial learning and memory in rats, suggesting a protective effect on hippocampal neurons.

It is well-established that hippocampal activity is modulated by a variety of neurotransmitters and neuromodulators (Khakpai et al., 2013). It has been demonstrated that the hippocampus is densely populated with cholinergic, glutamatergic, GABAergic, and monoaminergic axon terminals. These neurotransmitter systems are known to play critical roles in the encoding, storage, and expression of memory (Dani and Bertrand, 2007, Gulpinar and Yegen, 2004). Administration of aluminium chloride ($AlCl_3$) in rats resulted in a significant reduction in the levels of monoamines, including norepinephrine (NE), dopamine (DA), and serotonin (5-HT). Additionally, a significant disruption in the balance between glutamatergic and GABAergic signaling was observed, with $AlCl_3$ treatment causing an increase in hippocampal glutamate levels and a decrease in GABA concentrations. Furthermore, a marked increase in acetylcholinesterase (AChE) activity was detected in the hippocampal tissue of $AlCl_3$-treated rats, indicating enhanced acetylcholine metabolism. Similar alterations in neurotransmitter levels following $AlCl_3$ administration have been reported in previous studies with experimental animals. Notably, reductions in monoamine, acetylcholine (ACh), and GABAergic transmission, along with glutamatergic excess, have also been observed in humans with cognitive disorders, including AD (Chen et al., 2011). Agmatine dose-dependently mitigated aluminium chloride ($AlCl_3$)-induced neurochemical alterations and facilitated recovery in cholinergic, monoaminergic, GABAergic, and glutamatergic neurotransmitter systems, while reducing their metabolism in the hippocampal region of the rat brain. Therefore, the restoration of hippocampal neurotransmission may be a key factor contributing to the observed cognitive improvements following agmatine treatment in $AlCl_3$-treated rats.

The hippocampus is known to play a critical role in learning and memory processes. Aluminium chloride ($AlCl_3$) has been shown to induce damage to brain tissues, which are particularly susceptible to oxidative stress, mitochondrial dysfunction, and neuroinflammation (García-Escudero et al., 2013). Several studies have suggested that inflammation and oxidative stress play pivotal roles in the progression of aluminium chloride ($AlCl_3$)-induced neurotoxicity and neuronal cell death. In the present study, $AlCl_3$ administration resulted in a significant increase in oxidative stress markers, including malondialdehyde (MDA) and nitrite levels, while concurrently decreasing glutathione levels. Previous research has indicated that free radicals are highly effective at inducing neuronal cell death and may be involved in the pathogenesis of neurodegenerative diseases (Perry et al., 2002, Ischiropoulos and Beckman, 2003). Numerous studies have documented that aluminium chloride ($AlCl_3$) induces mitochondrial dysfunction, which subsequently leads to the generation of free radicals and oxidative damage (Kawahara and Kato-Negishi, 2011). The results suggest that aluminium chloride ($AlCl_3$) induces mitochondrial oxidative damage through increased oxidative

stress and decreased antioxidant levels. Treatment with agmatine significantly and dose-dependently mitigated $AlCl_3$-induced oxidative stress and restored the levels of antioxidant enzymes, such as glutathione (GSH), indicating its antioxidant properties. Several studies have demonstrated that agmatine possesses both antioxidant and anti-inflammatory effects in various experimental models. The findings of the present study imply that the improvement in cognitive function observed in $AlCl_3$-treated rats following agmatine administration may be attributed to its antioxidant and neuromodulatory activities.

## 5. Conclusions:

In conclusion, administration of aluminium chloride ($AlCl_3$) via oral gavage (p.o.) resulted in significant impairments in learning and memory, which were associated with altered brain neurochemistry, increased oxidative stress, and neuroinflammation. Agmatine treatment, in a dose-dependent manner, attenuated $AlCl_3$-induced cognitive deficits, oxidative stress, neuroinflammation, and restored brain neurochemical balance. The observed cognitive improvement in $AlCl_3$-treated rats following agmatine administration may be attributed to its antioxidant and anti-inflammatory properties, as well as its ability to modulate hippocampal neurochemistry. These findings suggest that agmatine could serve as a potential therapeutic candidate for the management of cognitive disorders, including Alzheimer's disease.

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## Author Contributions

Vandana Bhatia, Anjali Chandel conceptualized the Research Paper, designed the structure of the manuscript, and led the writing process. Priyanka Kashyap, Dr. Swati Rana, & Dheeraj conducted an extensive literature review, contributed to the writing of the various section in manuscript, and assisted in refining the manuscripts framework. Dr. Vir Vikram provided critical revisions and feedback, ensuring the accuracy and scientific integrity of the content. All authors participated in reviewing and editing the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

## Conflicts of interest

“The authors declare that they have no conflicts of interest.”

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