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Research Article

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Reversal of Aluminium chloride induced neurobehavioral deficit by Selenium: Involving NLRP3 inflammasome and cholinergic innervation

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Abstract

This study examines the efficacy of selenium as a therapeutic agent for reversing neurobehavioral deficits. A docking analysis was conducted to elucidate the interaction between selenium and the NLRP3 inflammasome residue. The research involved synthesizing and validating selenium nanoparticles within a controlled experimental design that employed Aluminium chloride ($AlCl_3$) to induce neurotoxicity, followed by comprehensive neurobehavioral evaluations in Wistar rats. The results revealed that a one-month oral selenium administration led to significant enhancements in cognitive and motor functions, reduced proinflammatory cytokine levels, and decreased β-amyloid aggregation within the brain. Quantitative findings demonstrated that selenium treatment led to a marked enhancement in locomotor activity (from 54.83±0.60 to 60.5±0.76 counts, $p<0.0001$), improved performance within the radial arm maze (with baited arm entries rising from 9.83±0.58 to 16.5±0.43, $p<0.0001$). They augmented the recognition index in the novel object recognition assessment (from 33.77±0.74% to 61.38±1.10%, $p<0.0001$). On a biochemical level, selenium was found to decrease malondialdehyde concentrations and elevate antioxidant enzyme levels (SOD, CAT, GSH, $p<0.01$) while also significantly reducing levels of β-amyloid and TNF-α in the brain tissue. Additionally, selenium exhibited neuroprotective properties through both antioxidant mechanisms and cholinesterase pathways, thereby establishing it as a promising candidate for treating neurobehavioral deficits and neuroinflammation.

Keywords: AlCl3; Docking study; Neurobehavioral; NLRP3; Selenium.

1. Introduction

Degenerative brain disorders affect millions of individuals worldwide. These neurodegenerative conditions are distinguished by the progressive decline of neurons that display selective susceptibility. They interfere with essential physiological functions, such as proteotoxic stress, oxidative damage, programmed cell death, and neuroinflammation, worsening neuronal dysfunction [1]. The diagnosis of Alzheimer's disease, Parkinson's disease, dementia, and a range of other disorders can be established by examining their primary clinical features.

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Alzheimer's disease is a particular type of dementia that is distinguished by pronounced memory loss and challenges in maintaining attention. The decline in cognitive abilities and memory occurs progressively over time. A range of unique characteristics identifies this disorder. As of 2015, around 44 million people globally had been diagnosed with Alzheimer's, and forecasts suggest that this figure may rise to 115 million by 2050 [1]. Alzheimer's disease (AD) is characterized by considerable brain tissue atrophy, with prominent effects on the cerebral cortex, parietal lobe, and frontal lobes. This neurodegenerative disorder is also defined by the presence of neurofibrillary tangles (NFT) and amyloid plaques. The accumulation of beta-amyloid (Aβ) is implicated in neurotoxic effects and increased oxidative stress, factors that are thought to contribute to AD's progression. While the exact causes of Alzheimer's disease are not fully understood, some research suggests a possible link to aluminum exposure.

Additionally, the disease manifests as increased oxidative stress, diminished acetylcholine (ACh) levels, and heightened activity of acetylcholinesterase (AchE) within the brain, alongside the accumulation of neurofibrillary tangles and amyloid plaques [2]. Acetylcholinesterase inhibitors, including tacrine, donepezil, and rivastigmine, enhance the levels of acetylcholine at synaptic sites and have shown effectiveness in enhancing cognitive abilities in patients diagnosed with Alzheimer's disease (AD). The blockade of acetylcholinesterase remains a significant area of interest for therapeutic strategies to manage dementia [3].

Aβ triggers the activation of the NLRP3 inflammasome within microglial cells via a newly identified signaling pathway. In experiments conducted using a rat model of Alzheimer's disease, the suppression of NLRP3 significantly alleviated spatial memory deficits and reduced Aβ accumulation. In contrast, the activation of NLRP3 is crucial to the progression of Alzheimer's disease, as it plays a pivotal role in fostering a harmful inflammatory response [4].

Aluminium has been associated with the development of Alzheimer's disease and is identified as a significant neurotoxin. Aluminium accumulation in the brain can disrupt mitochondrial function, increase the production of reactive oxygen species (ROS), and lead to mutations in DNA and proteins. Extended exposure to aluminum can cause neuronal damage and oxidative stress, both of which are considered contributing factors to the emergence of Alzheimer's disease [5]. Research has shown that aluminum supplementation triggers the activation of the NLRP3 inflammasome in macrophages. In experimental models, mice deficient in NLRP3, ASC, or caspase-1 fail to generate IL-1β when exposed to aluminum adjuvants [6].

The significance of selenium (Se) as a vital nutrient for humans and animals has attracted considerable attention in recent years. Studies indicate that Se is essential within the active site of glutathione (GSH) and is involved in numerous physiological and biochemical functions. These include the synthesis of coenzyme Q, the regulation of ion transport across cell membranes, the preservation of keratin structure, and the stimulation of antibody production. The protective effects of Se are primarily linked to its incorporation into selenoenzymes, which have been demonstrated to protect DNA and various cellular components from oxidative damage [7].

This study explores selenium's molecular and biochemical roles in mitigating the neurotoxic impacts caused by aluminum chloride ($AlCl_3$). The research will focus on the behavioral, biochemical, and molecular changes that $AlCl_3$ provokes in the brains of rats.

2. Materials and Methods

2.1. Chemicals and Reagents

For this investigation, an analytical grade of aluminum chloride was obtained from the Research Lab Fine Chem Industry. This aluminum chloride was subsequently dissolved in a phosphate-buffered saline (PBS) solution, which was prepared by combining 100 ml of distilled water with 800 mg of sodium chloride (NaCl), 144 mg of disodium hydrogen phosphate, 20 mg of potassium chloride, and 27 mg of potassium dihydrogen phosphate. The pH of the resulting solution was meticulously adjusted to 7.4, with the PBS being sourced from Loba Chemicals. Furthermore, selenious acid, ascorbic acid, and polyethylene glycol (PEG) of analytical grade were also acquired from Loba Chemicals.

2.2. Animals

A total of thirty male Wistar albino rats, with individual weights ranging from 180 to 250 grams, were employed in this research. The animals were accommodated in

polypropylene cages featuring standard lighting and ventilation, maintained under a 12-hour light/dark cycle at a controlled temperature of 25±3 °C and humidity levels between 35% and 60%. They were provided unrestricted tap water access and a standard pelletized diet. All pharmacological experimental procedures received approval from the Institutional Animal Ethics Committee under protocol number (CCSEA/IAEC/PT-01/02-2K23).

Table 1. Validation Parameters of the NLRP3 Protein Structure (PDB ID: 7ALV)

Parameters	Details	Standards
Target	NLRP3	
Protein Id	7ALV	-
Method of experiment	X-RAY Diffraction	X-RAY Diffraction
Mutation	No	No
Resolution	2.83 Å	Near about 3.00 Å
wwPDB Validation	Better	Better
Co-Crystal Ligand	RM5	-
Ramachandran Plot (by PROCHECK server) Residues in favored + Allowed regions	92.4 %	>88 %

2.3 Molecular Docking Studies

2.3.1. Target Protein Retrieval and Preparation

The X-ray crystallographic structures of NLRP3 (7ALV) were sourced from the RCSB Protein Data Bank. This structural data was subjected to a comprehensive validation process involving various parameters, such as resolution assessment, mutation analysis, wwPDB criteria, co-crystal ligand evaluation, and Ramachandran plot examination. The details of this validation methodology are provided in the following sections and in **Table 1.**

2.3.2. Grid Generation

Auto Dock Tools version 1.5.65, Chimera version 1.112, and Maestro version 12.7.1619 were utilized to generate and validate grids. The parameters for the grids were determined based on the orientation of the co-crystal ligand, or the CASTp6 server was employed, provided that the protein was in its apo conformation. The amino acid residues that are specifically involved in the active site of NLRP3, which were utilized for grid generation, are given in **Table 2**

Table 2. Active Site Amino Acids of the NLRP3 Protein (PDB ID: 7ALV)

Protein ID	Active Sites of Amino Acids
7ALV	ALA227A, ALA228A, ARG351A, PRO352A, MET408A, PHE410A, ILE411A, THR439A, THR524A, ILE574A, PHE575A, ARG578A, LEU628A, GLU629A, TYR632A, MET661A

2.3.3. Ligands Preparation

Ligand extraction was performed using ChemSpider3, followed by the importation of the compound into MarvinSketch4 for both 2D and 3D structural refinement. The refined structures were minimized using the MMFF94 force field, from which the conformer with the lowest energy was selected for further analysis in MOL2 format [8].

2.3.4. Molecular Docking of Target Protein with Ligands

The ligands and proteins were acquired, and the resulting structures were transformed into pdbqt format using AutoDock Tools version 1.5.6. We utilized AutoDock Tools 1.5.6 for our docking experiments, establishing a grid point spacing of 0.375 Å. The grid box was intentionally located over the target's active site, allowing the software to investigate further potential interactions between the ligands and the receptor. Following the preparation for docking, the Cygwin terminal was employed to carry out the docking procedure [9].

2.3.5. Visualization:

The results derived from the Autodock Vina assessment were employed to build a complex utilizing the Biovia Discovery Studio visualizer. Furthermore, Maestro 12.3 (academic version) was instrumental in creating two-dimensional and three-dimensional representations of the complex [10].

2.4. Formulation & Development

In the preliminary phase, precise amounts of the precursor, selenous acid, and the stabilizing agent, Polyethylene glycol, were accurately measured and dissolved in 100 mL of distilled water. Subsequently, an ascorbic acid solution was prepared by dissolving the acid in 50 mL of distilled water. During the final phase of the synthesis, the ascorbic

acid solution was swiftly added to the mixture containing the previously measured quantities of the precursor and stabilizer while ensuring vigorous stirring, with the combined solution being agitated for 5 to 10 minutes. A multifactorial experimental design was applied to optimize the selenium synthesis methodology, which included three input parameters, each investigated at three different levels. The selenous acid concentrations utilized were 0.05, 0.08, and 0.15 M, while ascorbic acid concentrations were established at 0.0332, 0.0421, and 0.5 M. [11].

2.5. Experimental design

Wistar rats were assigned to different treatment groups prior to the initiation of the experiment. As a result, four distinct groups were formed, each consisting of six rats (n=6). The dosage administration was determined according to the rats' body weight (B.W.), specifically those within the 200 to 250 grams range. The rats were given a standard diet and housed in cage racks with controlled humidity and temperature (12-hour cycles, 25±3 °C, 35–60% humidity). Group 1 acted as the control group, receiving saline (0.1% NaCl) once daily for 90 days. Groups 2 to 4 were given Aluminum chloride (100 mg/kg) orally once daily for 60 days. On the 61st day, Group 3 was administered Donepezil (10 mg/kg) orally, while Group 4 received Selenium (5 mg/kg) through the oral route.

Daily monitoring of the rats' dietary intake and weight fluctuations was conducted throughout the study. Before their euthanasia at the experiment's end, each rat underwent a series of following behavioral evaluations. After applying anesthesia, blood and brain tissue samples were obtained for later pathological and biochemical examination.

2.6. Neurobehavioral Testing

2.6.1. Actophotometer

The locomotor activity of the animals was evaluated through the use of an Actophotometer. Each animal was placed within the Actophotometer, and an initial activity score was recorded over five minutes. After administering the appropriate medication to each animal, a follow-up activity score was taken after 20 minutes. A decrease in the activity score was identified as a significant marker. Furthermore, the latency time of the rat was assessed and employed as a neurobehavioral metric [12].

2.6.2. Radial arm maze (RAM)

The RAM test was conducted with slight alterations to the initial protocol. In order to increase participant engagement, the experimental rats were placed on a regulated diet. Various visual extra-maze cues were intentionally arranged within the maze, set approximately 10-30 cm apart. Each arm of the maze was equipped with a circular feeding well at its end, measuring 1 cm in diameter and 0.5 cm in depth. On the first day, the rats were positioned at the center of the platform and supplied with food pellets. They were subsequently permitted to explore the baited arms over five days. Upon the conclusion of this phase, on the eighth day, the rats underwent a similar training protocol to evaluate their working and reference memory skills [13].

2.6.3. Novel object recognition

The study was conducted in a square open arena measuring 50 × 50 cm², enclosed by four walls, each standing 50 cm tall. Initially, each rat was given five minutes to familiarize itself with the empty environment. After a 24-hour pause, the rats participated in a five-minute "acquisition" phase, during which two identical objects (A1 and A2) were placed in adjacent corners, positioned 10 cm from the walls. Following another 24-hour interval, one of the objects (A1 or A2) was replaced with a novel object (B), and the rats were again allowed five minutes for exploration. The primary aim of the experiment was to assess the memory and recognition abilities of the rats by requiring them to distinguish between the familiar objects (A1, A2) and the new object (B) after the designated delay. The Recognition Index (RI) was then computed based on the data gathered from the subjects [14].

$$\%\ Recognition\ Index = \frac{Time\ spent\ by\ rat\ towards\ Novel\ object}{Total\ time\ spent\ by\ rat\ in\ NOR\ apparatus} \times 100$$

2.7. Biochemical Estimation

2.7.1. Tissue Preparation

The animals were weighed, and their vital organs were meticulously extracted. Each organ underwent a rinsing process twice using ice-cold tris buffer. Following the

recording of tissue weight, a 10% solution was formulated with tris-buffer (10 mM, pH 7.4). The tissue was then chopped into fine pieces and homogenized to achieve a consistent mixture. This homogenate was subsequently transferred into a plastic centrifuge tube and centrifuged at 6000 rpm for a duration of 20 minutes while being kept chilled. The final homogenate was preserved in a frozen state for subsequent analysis.

2.7.2. Thiobarbituric Acid Reactive Substances (TBARS)

The study utilized the methodology devised by Okawa et al. in 1979 to assess the concentrations of thiobarbituric acid reactive substances (TBARS) within brain homogenates. For sample preparation, the brain homogenates were treated with a mixture of 1.5 ml of acetic acid, 0.2 ml of 8.1% sodium lauryl sulfate, and 1.5 ml of thiobarbituric acid. The mixtures were then incubated for one hour at a temperature of 95°C, after which n-butanol was incorporated, and the samples were allowed to cool. Following this, the organic phase was extracted and centrifuged for five minutes, with absorbance readings taken at 532 nm using a UV spectrophotometer. The concentrations of TBARS were expressed as nmol/mg of protein [15].

2.7.3. Superoxidase dismutase

The assessment was conducted using brain homogenates. The evaluation of superoxide dismutase (SOD) activity was performed by assessing the inhibition of superoxide-mediated reduction. SOD activity was expressed in terms of units per milligram of protein (U/mg). A preparation contained 0.5 ml of homogenate, 0.5 ml of distilled water, 0.38 ml of ethanol, and 0.15 ml of chloroform. Subsequently, the supernatant was separated through centrifugation at 2000 rpm. 1.2 ml of the supernatant was used in one vial, while another vial contained 0.5 ml. An equal carbonate buffer solution (pH 10.2) was introduced into both vials. Furthermore, EDTA was added, with 0.5 ml included in the second vial and 1.2 ml in the first [16].

2.7.4. Catalase

Catalase (CAT) activity was assessed in this study by using brain homogenates. The CAT enzyme's activity was quantified in units per milligram of protein. The reaction mixture had a total volume of 3 ml, which included 2 ml of phosphate buffer at pH 7.0, 0.95 ml of hydrogen peroxide at a concentration of 0.019 M, and 0.05 ml of the supernatant. Absorbance measurements were recorded at 240 nm at 10-second intervals for a total duration of 1 minute. One unit of CAT was defined as the quantity of enzyme necessary to catalyze the decomposition of 1 µmol of hydrogen peroxide per minute at a temperature of 25°C. The results were reported as CAT U/g of brain tissue.[17].

2.7.5. Glutathione reductase

The activity was assessed in brain homogenates. Mix 0.5 ml of tissue homogenate with 0.1 ml of 10% trichloroacetic acid. Subsequently, centrifuge the resulting mixture at 2000 g for 10 minutes at a temperature of 4°C. The supernatant obtained from this process was utilized to measure GSH levels. To 300 µl of the processed tissue sample, incorporate 0.5 ml of phosphate buffer (0.1 M, pH 8.4) and 0.2 ml of DTNB. Vortex this mixture thoroughly. The absorbance was measured at 412 nm within 15 minutes. Results were presented as U/mg of protein [17].

2.7.6. Acetylcholinesterase assay

The activity was assessed in brain homogenates. A reaction mixture was formulated by combining 0.4 ml of brain homogenate, 2.6 ml of ice-cold phosphate buffer solution (0.1M, pH 8.0), and 100 µl of DTNB. This mixture was then subjected to air mixing before analysis using a spectrophotometer. Once the reaction stabilized, the initial absorbance was measured at 412 nm. Following this, 5.2 µl of Acetylcholine chloride was added to the mixture. The absorbance was continuously monitored from the initiation of the reaction for 10 minutes at a temperature of 25°C within the cuvette [17].

2.7.7. Beta-amyloid protein estimation

The study was conducted following established protocols, which required the creation of a standard curve for each assay and examining both standards and samples in triplicate. Before adding the pre-complexed standards, 2 µl of biotinylated BAPP antibody was dispensed into the sample wells. Afterward, Streptavidin: HRP Conjugate was added to each well, and the well was allowed to incubate at 37°C for 60

minutes. Once this incubation period concluded, the plates were washed four times before TMB Substrate was applied to each well, followed by an additional incubation of 10 minutes at 37°C [18].

2.7.8. TNF- alpha estimation

The reagents were equilibrated to ambient temperature before analysis, with both standards and samples assessed in duplicate. A high standard concentration of 2000 pg/ml was achieved by reconstituting a lyophilized vial in 730 µl of distilled water. The plates were thoroughly cleaned, dried by blotting, and then incubated in various solutions. The methodology included adding 100 µl of detection antibody, 100 µl of streptavidin-HRP, and 100 µl of TMB substrate. The reaction was terminated using a stop solution, and the absorbance was measured at 450 nm within 30 minutes [19].

2.9. Statistical analysis

Data were presented as mean ± standard deviation (SD) from the experiments. Statistical analyses were performed using GraphPad Prism version 10.0. The experimental groups underwent evaluation through ANOVA, followed by the Tukey multiple comparison test. A P-value of less than 0.0001 was considered indicative of statistical significance.

3. Results and Discussion

3.1. Molecular Docking

The docking analysis revealed that selenium is capable of binding to the NLRP3 protein (PDB ID: 7ALV) through the formation of multiple hydrogen bonds, particularly with key amino acids such as PHE568, GLU569, GLY309, GLN308, and LEU307. **Table 3** illustrates the strength of this interaction and identifies the specific regions of the protein that selenium interacts with. The binding energy measured was –1.97 kcal/mol, suggesting that selenium establishes a stable and specific interaction with NLRP3.

Figure 1 presents a visual representation of this interaction, generated with LigPlot+, which precisely indicates the binding sites of selenium on the protein. These findings imply that selenium might inhibit the activation of NLRP3, potentially elucidating its protective role against brain injury induced by aluminium chloride.

Table 3. Docking Score and intermolecular interactions of ligands with the Protein

Sr.No	Molecule	Binding Energy kcal/mol	Type of Interaction	Residue ID	Distance
1	Selenium	-1.97	Hydrogen bond	PHE568	3.3
				GLU569	3.19
				GLY309	2.82
				GLN308	3.87
				LEU307	3.23

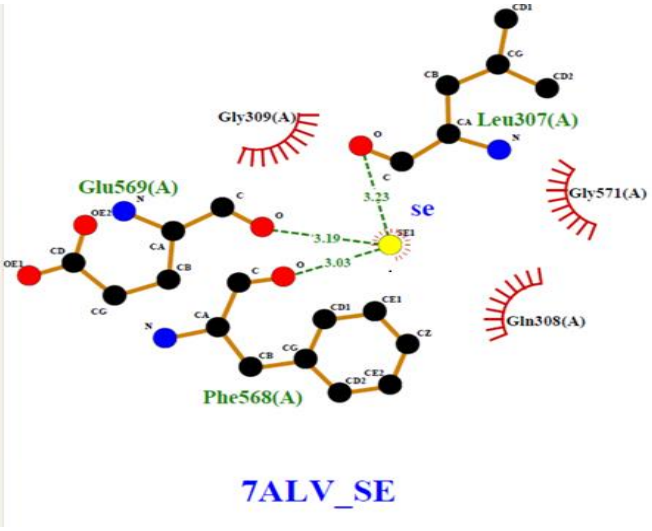

Figure 1: Ligplot of the interaction of the protein 7ALV with ligand Selenium using ligplus software.

3.2. Effects on Behavioural Parameters

3.1.1. Locomotor Activity by Actophotometer

The study demonstrated that exposure to $AlCl_3$ resulted in a marked reduction of locomotor activity in rats, achieving statistical significance ($p<0.0001$) when assessed against the control group during the 0-10 and 10-20 minute time frames. Additionally, the introduction of selenium appeared to alleviate the neurotoxic effects of $AlCl_3$, leading to a statistically significant enhancement in locomotor activity ($p<0.0001$) compared to the disease control group. These results were comparable to those recorded with the standard treatment involving donepezil (**Table 4**). The findings indicate that selenium could potentially aid in the recovery of locomotor coordination and activity levels compromised by neurotoxicity induced by aluminum, likely through the modulation of oxidative and cholinergic pathways.

Table 4: Effect of Selenium on Locomotor Activity in AlCl3-Induced Neurobehavioral Deficient Rat Model

Animal Groups	Locomotor activity counts in minutes	
	0-10 min	10-20 min
Control	66±0.733	70±0.579
Disease Control	54.83±0.603***	60.5±0.673***
Donepezil	63.66±0.805###	67±0.579###
Selenium	60.5±0.766###	66.33±0.592###

Results are expressed as Mean ± SEM (n=6). *** Indicates significantly ($p<0.0001$) different compared to control group; ### indicates significantly ($p<0.0001$) different compared to disease control. Comparisons between the groups were made using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons.

3.2. Radial Arm Maze

The study revealed that $AlCl_3$ substantially reduced the frequency of entries in the radial arm maze ($p<0.0001$) compared to the control group. Additionally, selenium was shown to significantly alleviate the neurotoxic effects of $AlCl_3$ and increase the number of entries compared to the disease control group ($p<0.0001$). These results were consistent with those noted in the standard treatment involving donepezil **(Table 5)**. The increase in baited arm entries signifies advancement in spatial learning and working memory, implying that selenium may protect hippocampal functionality impaired by $AlCl_3$.

Table 5: Effect of Selenium on Cognitive Performance Activity in $AlCl_3$-Induced Neurobehavioral Deficit Rat Model

Animal Groups	There are no entries in the arms	
	Baited Arm	Unbaited Arm
Control	19.16±0.703	9.83±0.479
Disease Control	9.83±0.587***	4.66±0.456***
Donepezil	18±0.579###	10±0.518###
Selenium	16.5±0.429###	8.33±0.496###

Results are expressed as Mean±SEM (n=6). *** Indicates significantly ($p<0.0001$) different compared to control group; ### indicates significantly ($p<0.0001$) different compared to disease control. A group comparison was made using one-way ANOVA analysis of variance (ANOVA) followed by multiple Tukey comparisons.

3.3. Novel Object Recognition

The study's results indicated that AlCl3 substantially ($p<0.0001$) compromised neurobehavioral functions, as reflected by a decrease in the % Recognition Index compared to the control group of rats. In contrast, the introduction of Selenium notably ($p<0.0001$) alleviated the neurotoxic effects associated with $AlCl_3$, improving the % Recognition Index when analyzed against the disease control group. These findings were analogous to those obtained with the established treatment using donepezil **(Table 6)**. The elevated recognition index indicates improved memory retention and cognitive flexibility, thereby reinforcing the neuroprotective function of selenium in counteracting memory deficits induced by AlCl3.

Table 6: Effect of Selenium on Novel Object Recognition in AlCl3-induced neurobehavioral deficit rat model

Animal Groups	% Recognition Index
Control	69.48±1.55
Disease Control	33.77±0.74***
Donepezil	63.7±1.24###
Selenium	61.38±1.10###

Results are expressed as Mean±SEM (n=6). *** Indicates significantly ($p<0.0001$) different compared to control group; ### indicates significantly ($p<0.0001$) different compared to disease control. A comparison between the groups was made using a one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons.

3.4. Effects on Antioxidant Parameters

To assess the impact of Selenium on oxidative stress, we measured the levels of malondialdehyde (MDA), a product of lipid peroxidation, alongside the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in brain tissue samples from both control and treatment groups **(Figure 2)**. The MDA concentration in rats subjected to $AlCl_3$ (Disease control) was significantly elevated ($p>0.01$) compared to that in control group I. Conversely, treatment with Selenium (5 mg/kg) resulted in a marked reduction in MDA levels when compared to the $AlCl_3$-induced group ($p>0.01$). Additionally, the rats in the $AlCl_3$ treatment group displayed notably lower levels of SOD, CAT, and GSH ($p>0.01$) relative to the control group. In contrast, administration of Selenium Nanoparticles led to a significant increase in these antioxidant enzyme levels ($p>0.01$) compared to the $AlCl_3$-induced rats (Disease control). This investigation demonstrates that Selenium (5 mg/kg) possesses antioxidant capabilities and can mitigate oxidative damage induced by $AlCl_3$ in rats. These results indicate that selenium significantly strengthens the brain's antioxidant defense mechanism, which may help avert neuronal damage associated with Alzheimer's disease caused by oxidative stress.

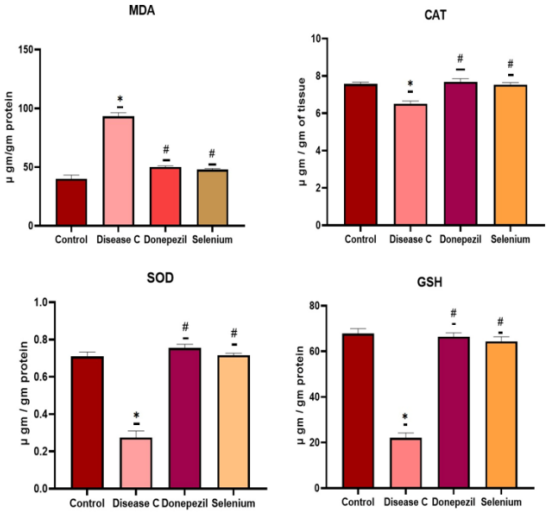

Figure 2: Effect of Selenium on MDA, CAT, SOD, and GSH in $AlCl_3$-induced rat model.
Results are expressed as Mean ± SEM (n=6). * Indicates significantly (p<0.01) different compared to control group; # indicates significantly (p<0.01) different compared to disease control. Comparisons between the groups were made using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons.

3.5. Effect on β-amyloid protein activity

The findings indicate that $AlCl_3$ significantly elevates β-amyloid protein levels ($p < 0.01$) compared to the vehicle control. Furthermore, selenium substantially mitigated the neurotoxic effects of $AlCl_3$ ($p < 0.01$) and reduced β-amyloid protein levels. These outcomes were comparable to those observed with the standard treatment involving donepezil. These results indicate that selenium may play a role in reducing amyloidogenic pathways associated with Alzheimer's disease, potentially by disrupting Aβ aggregation or its inflammatory effects **(Figure 3)**.

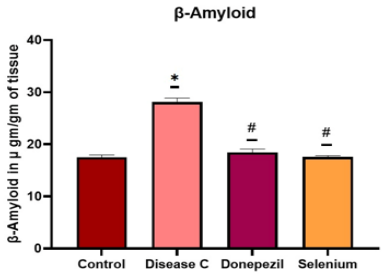

Figure 3. Effect of Selenium on β-amyloid protein in $AlCl_3$ induced rat model.
Results are expressed as Mean±SEM; (n=6). * Indicates significantly (p<0.01) different compared to control group; # indicates significantly (p<0.01) different compared to disease control. Comparisons between the groups were made using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons.

3.6. Cholinesterase Enzyme Assay

The findings indicated that $AlCl_3$ significantly elevated acetylcholinesterase levels ($p < 0.01$) compared to the vehicle control group. Furthermore, selenium notably mitigated the neurotoxic impact of $AlCl_3$ ($p<0.01$) and reduced AchE levels. These results were similar to those observed with the standard treatment of donepezil. The decrease in acetylcholinesterase levels suggests that selenium may assist in reinstating cholinergic neurotransmission, thereby enhancing cognitive function impaired by exposure to $AlCl_3$ **(Figure 4)**.

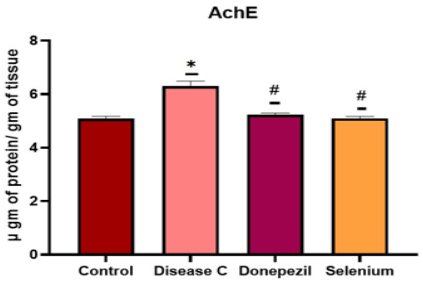

Figure 4: Effect of Selenium on AchE in $AlCl_3$ induced rat model.

Results are expressed as Mean±SEM; (n=6). * Indicates significantly (p<0.01) different compared to control group; # indicates significantly (p<0.01) different compared to disease control. Comparisons between the groups were made using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons.

3.7. Effect on TNF-α level (Inflammatory mediator)

The findings indicated that treatment with $AlCl_3$ led to a substantial increase in TNF-α levels ($p<0.01$) compared to the vehicle control group. Furthermore, selenium exhibited a significant protective effect against the neurotoxic impact of $AlCl_3$, resulting in a notable reduction in TNF-α levels ($p<0.01$). These results were similar to those observed with the administration of donepezil. The decrease in TNF-α indicates that selenium has anti-inflammatory effects, which might assist in combating neuroinflammation, a key factor in advancing Alzheimer's disease **(Figure 5)**.

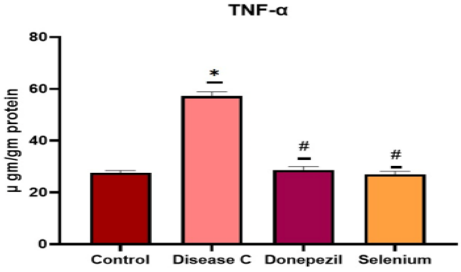

Figure 5. Effect of Selenium on TNF-α level in $AlCl_3$ induced rat model.
Results are expressed as Mean±SEM; (n=6). * Indicates significantly (p<0.01) different compared to control group; # indicates significantly (p<0.01) different compared to disease control. Comparisons between the groups were made using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons.

3.8. Histopathological Analysis

The research indicates that the oral administration of Selenium influences the deterioration of brain tissue in the context of disease control. Administration of Selenium at a dosage of 5mg/kg did not produce significant degeneration in brain tissue when contrasted with the rats in the disease control cohort. The control group of rats exhibited typical brain histopathology, characterized by granular, pyramidal, and molecular cells within the cerebellar layers. Furthermore, Selenium treatment may play a role in mitigating neuronal damage in rats induced by $AlCl_3$ (**Figure 6**).

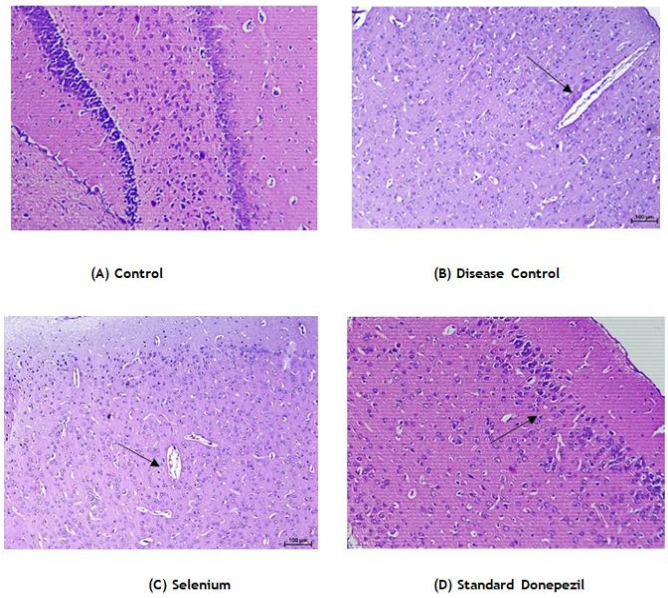

Figure 6. Histopathological changes in aluminum chloride ($AlCl_3$)-induced rats.

3.9. Discussion

Alzheimer's disease is fundamentally marked by the buildup of amyloid-beta (Aβ). A substantial body of research has demonstrated that Aβ can stimulate the NLRP3 inflammasome within microglial cells, leading to neuroinflammatory reactions. Investigations by Akama and collaborators showed that Aβ fibrils trigger the activation of the NLRP3 inflammasome, which in turn enhances the synthesis of interleukin-1 beta (IL-1β) in cultured glial cells. Moreover, interventions aimed at Aβ were observed to diminish the expression levels of NLRP10, which serves as a negative regulator of the NLRP3 inflammasome [20].

In this research, we performed molecular docking analyses to investigate the connection between molecular docking and the biological efficacy of selenium nanoparticles. The molecular docking approach enables a swift evaluation of the interactions between the target protein and ligand binding, which are pivotal for biological functionality. Our research concentrated on the specific interactions between selenium and the NLRP3 target. The results demonstrate that selenium establishes hydrogen bonds with five binding residues of the NLRP3 protein (PDB ID: 7ALV), resulting in a docking score of -1.97. These findings imply a strong binding affinity of selenium to the NLRP3 site, with the docking score as an indicator of the binding free energy. Given these observations, we intend to optimize selenium formulation to enhance its biological activity.

Selenium plays a protective role for the brain by combating detrimental molecules known as reactive oxygen species (ROS), which can potentially harm brain cells. It accomplishes this by integrating into specific proteins, such as glutathione peroxidase and thioredoxin reductase, that assist in maintaining the brain's chemical equilibrium. Furthermore, selenium mitigates immune responses by inhibiting certain signals (such as NF-κB) and alleviating inflammation triggered by the NLRP3 inflammasome. Given its capacity to diminish oxidative stress while regulating inflammation, selenium is particularly crucial for neurological disorders like Alzheimer's disease, where both factors significantly contribute to the damage of nerve cells. [21]

Oxidative damage has been widely associated with various neurodegenerative diseases, particularly Alzheimer's disease (AD). Aluminium chloride ($AlCl_3$) has been linked to the development of neurological disorders, notably Alzheimer's disease. The neurotoxic properties of metals are mainly due to their ability to provoke oxidative stress. Additionally, $AlCl_3$ presents considerable hazards since it can enter the food chain through compromised drinking water and dietary sources [22]. Aluminum chloride ($AlCl_3$) can traverse the blood-brain barrier (BBB) and tends to accumulate within the hippocampus, an area essential for cognitive functions such as memory and learning. Prolonged aluminium accumulation (Al) is associated with neurotoxic consequences, manifesting as neurofibrillary tangles and amyloid plaques. Numerous studies have reported elevated concentrations of aluminum in the brains of patients diagnosed with Alzheimer's disease.

$AlCl_3$ triggers oxidative stress, resulting in the buildup of nerve fibers and demonstrating neurotoxic properties. Previous research has suggested that selenium may protect against Alzheimer's disease [23].

Examining the underlying mechanisms of neurotoxicity caused by $AlCl_3$ is crucial, especially in light of the lack of effective therapies for Alzheimer's disease. Previous studies utilizing animal models have demonstrated that exposure to $AlCl_3$ results in memory and learning deficits in rats, which are linked to neurochemical alterations. The current research sought to evaluate the impact of selenium on rats exposed to $AlCl_3$. The rats received oral doses of $AlCl_3$ to analyze its effects on neurobehavioral outcomes. Findings indicated a notable decrease in locomotor activity, as assessed by the Actophotometer, along with impairments in cognitive functions such as learning and memory, highlighted by performance variations in the Radial arm maze and reduced recognition abilities in the Novel object recognition test. These findings reflect prominent symptoms associated with Alzheimer's disease. Importantly, the administration of selenium seemed to alleviate these adverse effects, thereby improving the rats' spatial memory and learning functions.

The noted enhancement in the activities of antioxidant enzymes (SOD, CAT, GSH) within brain tissue after selenium treatment aligns with the strategies utilized for biochemical assessment through brain homogenates. Likewise, the behavioral data obtained through the actophotometer and radial maze corroborate the functional results of selenium's molecular mechanisms, as evident in docking studies.

The brain is particularly susceptible to oxidative stress because of its high rate of oxygen usage, extended cell division periods, and comparatively low concentrations of antioxidants. When exposed to aluminum, the brain can experience considerable oxidative damage, which may cause injury to neurons and interfere with the brain's antioxidant protective mechanisms [24]. The imbalance between oxidative damage and antioxidant defenses is linked to immediate memory impairments and may act as an early marker for the development of Alzheimer's disease. This research demonstrated that aluminum exposure led to oxidative stress, as indicated by increased malondialdehyde (MDA) levels in rats that received $AlCl_3$, in contrast to the control group. These results are consistent with findings from prior studies [25]. Selenium therapy resulted in a substantial decrease in MDA levels compared to animals subjected to $AlCl_3$ induction. Conversely, rats treated with $AlCl_3$ demonstrated significantly diminished levels of SOD, CAT, and GSH relative to the control group [26]. The administration of Selenium led to a significant increase in levels relative to rats exposed to $AlCl_3$ induction. This study suggests that Selenium demonstrates antioxidant properties and may mitigate oxidative damage caused by $AlCl_3$ in rat models.

Amyloid deposits in the hippocampus, amygdala, and neocortex are important histological markers for Alzheimer's disease (AD). The literature also emphasizes the impact of $AlCl_3$ on this pathology. The study revealed that $AlCl_3$ significantly increased the levels of β-Amyloid protein compared to the vehicle control group. Additionally, selenium treatment alleviated the neurotoxic effects attributed to $AlCl_3$ and reduced the levels of β-Amyloid protein [27].

The cholinergic nerve system has a role in both learning and memory. Acetylcholine, a cholinergic neurotransmitter, is subject to breakdown by the enzyme acetylcholinesterase (AchE). Disruption in cholinergic signaling has been linked to memory impairments characteristic of Alzheimer's disease. Aluminium is noted for its role as a potent cholinotoxin, capable of crossing the blood-brain barrier (BBB), thus interfering with cholinergic communication. Studies have demonstrated that administering aluminum chloride ($AlCl_3$) in rat models significantly increases AchE levels. In contrast, selenium supplementation has been shown to alleviate the neurotoxic effects associated with $AlCl_3$ exposure in rats, corresponding with a decrease in AchE levels. This finding suggests that selenium may offer neuroprotective properties by inhibiting AchE activation.

Alzheimer's disease begins with inflammatory responses that are crucial to its pathophysiology. In neurodegenerative conditions such as Alzheimer’s, the damage to neurons prompts the secretion of proinflammatory cytokines in targeted areas of the brain. Among these cytokines, TNF-α has been identified as a key player in the induction of neuroinflammation [28]. The current investigation identified a notable increase in

TNF-α levels in rats exposed to $AlCl_3$, consistent with previous studies regarding the effects of $AlCl_3$ exposure. Furthermore, the introduction of Selenium led to a significant reduction in the proinflammatory cytokine TNF-α, indicating its possible role in suppressing the signaling mechanisms of inflammatory mediators. Taken together, these findings suggest that Selenium treatment may serve as an effective therapeutic strategy for managing $AlCl_3$-induced Alzheimer's disease in rat models.

4. Conclusion

In conclusion, Selenium significantly mitigates neurotoxicity caused by $AlCl_3$, as evidenced by enhancements in locomotor performance, learning capabilities assessed through the radial arm maze model, and recognition skills evaluated in the novel object recognition test model.

In the $AlCl_3$ model, the suggested mechanism underlying the neuroprotective effects of Selenium may involve a decrease in beta-amyloid protein levels, a reduction in cholinergic activity, a lower concentration of the proinflammatory cytokine TNF-alpha, and an enhancement of antioxidant capacity. These findings indicate that Selenium could be a promising therapeutic agent for neurodegenerative diseases. Nevertheless, further research is necessary to validate the anti-Alzheimer properties of Selenium across various Alzheimer's disease models prior to initiating clinical trials.

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Conflict of interest

The authors stated that all relevant conflicts of interest and affiliations are disclosed. There are no financial conflicts of interest in the present manuscript work.
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Data availability

The authors confirm that the data supporting the findings of this study are available within the article. The raw data that support the findings of this study are available from the corresponding author on request.

Authors Contributions

SVT, substantially contributes to the conception, design, and supervision of the work, approved the final version for publication, and agreed to be accountable for all aspects of the work.
AD, substantially contributed to the acquisition, experimental, analysis, and interpretation of the data, drafted the manuscript, critically revised it for important intellectual content, approved the final version for publication, and agreed to be accountable for all aspects of the work.
SG, contributed to the analysis, manuscript editing, critical revision for intellectual content and agreed to be accountable for all aspects of the work.
SD, contributed in designing of the molecular modeling study, interpreted the results and approved the final version for publication
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There was no use of artificial intelligence in the making of this article.

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