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Polyphenol Alleviation of Aluminum Chloride-Induced Cognitive Impairment and Synaptosomal Ca^{2+} Homeostasis in Rats

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Abstract: Background: Alzheimer's disease (AD) is driven by convergent mechanisms that include oxidative stress and Ca^{2+}-dependent synaptic failure [1–4]. Aluminum chloride ($AlCl_3$) exposure is frequently used to reproduce selected AD-like features in rodents, including cognitive/behavioral decline and redox imbalance [7–9]. Here, dissertation-derived experimental results are reformatted into an IMRAD manuscript to assess whether a plant-derived polyphenol fraction (G-31) can correct $AlCl_3$-evoked behavioral suppression and synaptosomal Ca^{2+} dysregulation.

Methods: Male white rats (180–200 g) were assigned to control and $AlCl_3$ model groups; $AlCl_3$ was administered

(10 mg/kg, i.p., once daily, 7 days) to induce AD-like neurotoxicity. G-31 was given at 50 mg/kg using different delivery routes (i.p., intranasal, or per os; n=6/group). Behavior was quantified by open-field exploration (42-square arena, 3 min) [5], Conditioned reflex passive avoidance (CRPA) and Conditioned reflex active avoidance (CRAA). Synaptosomes were prepared by differential centrifugation and loaded with Fluo-4AM to quantify cytosolic Ca^{2+} kinetics (peak amplitude, AUC, τ) under Ca^{2+}-containing (2 mM $CaCl_2$) or Ca^{2+}-free (EGTA) conditions. Oxidative stress was evaluated by malondialdehyde (MDA) in blood and brain homogenates.

Results: The $AlCl_3$ model robustly increased lipid peroxidation: MDA rose from 11.4±0.1 to 30.2±0.3 µmol/mg tissue in blood and from 4.54±0.4 to 8.35±0.2 µmol/mg tissue in brain ($p<0.05$–0.01). $AlCl_3$ exposure also produced a hypomotor/exploratory phenotype in the open field and decreased performance in avoidance-based cognitive paradigms. At the synaptic level, synaptosomal Ca^{2+} transients deviated from the control pattern, consistent with Ca^{2+} dyshomeostasis—an established mechanistic hallmark of AD-related synaptic vulnerability [10–12]. Across regimens, G-31 shifted behavioral and Ca^{2+} readouts toward the control profile; intranasal delivery produced the most pronounced behavioral correction in this dataset.

Conclusion: These results support a working model in which $AlCl_3$ triggers oxidative membrane injury and synaptosomal Ca^{2+} dysregulation that jointly contribute to cognitive suppression, and polyphenol G-31 provides partial, multi-level correction—potentially via antioxidant/metal-chelating effects and normalization of Ca^{2+} entry/clearance mechanisms [14–19].

Keywords: Aluminum chloride; Alzheimer-like model; polyphenol; synaptosomes; calcium dynamics; oxidative stress; open-field; passive avoidance.

Introduction: Alzheimer's disease (AD) is a leading cause of dementia and is pathologically characterized by amyloid-β (Aβ) aggregation, tau pathology, synapse loss and neuroinflammation [1,2]. Although Aβ and tau remain core hallmarks, a substantial body of work indicates that oxidative stress and disturbed intracellular Ca^{2+} signaling provide a convergent mechanistic pathway driving synaptic failure and neuronal vulnerability [3,4,10–12]. At synapses, Ca^{2+} is the central second messenger coordinating neurotransmitter release, long-term potentiation (LTP), and activity-dependent gene expression. Accordingly, even moderate shifts in Ca^{2+} influx/clearance balance can impair plasticity programs and amplify excitotoxic and inflammatory cascades.

Aluminum exposure has long been investigated as a potential contributor to neurotoxicity. In rodent studies, aluminum salts can increase oxidative stress, impair mitochondrial bioenergetics, and disrupt signaling pathways relevant to learning and memory [7–9]. In particular, $AlCl_3$ has been shown to impair long-term memory and downregulate the cAMP–PKA–CREB axis, which is required for memory consolidation [8]. Therefore, $AlCl_3$-based paradigms are useful for testing candidate neuroprotectants and for linking behavioral phenotypes to molecular readouts.

Polyphenols are promising multi-target compounds for CNS disorders. Reported mechanisms include direct scavenging of reactive oxygen species (ROS), chelation of redox-active metals, rebalancing of pro-inflammatory transcriptional programs (e.g., NF-κB), and facilitation of synaptic plasticity mediators (e.g., BDNF/CREB signaling) [14–19]. However, for translational relevance, behavioral rescue should be anchored to mechanistic endpoints at the synaptic level.

This manuscript converts dissertation-derived findings into an IMRAD article focusing on (i) $AlCl_3$-induced oxidative stress and behavioral suppression, (ii) synaptosomal Ca^{2+} dysregulation as a mechanistic marker, and (iii) the corrective profile of polyphenol fraction G-31 across delivery routes.

METHODS

Animals and experimental groups. Male white rats (180–200 g) were maintained under standard vivarium conditions. Animals were distributed into experimental groups (n=6/group). AD-like neurotoxicity was induced by $AlCl_3$ (10 mg/kg, intraperitoneally, once daily for 7 consecutive days). The polyphenol fraction G-31 was administered at 50 mg/kg via i.p., intranasal, or per os routes (Table 1).

Open-field testing. Exploratory activity was assessed in a 42-square arena with holes under ~100-lux illumination. Following a 2–3 min acclimation, animals were monitored for 3 min. Endpoints included horizontal locomotion (square crossings), vertical activity (rearing), grooming, hole pokes, and emotionality indices (defecation/urination) [5].

Conditioned reflex passive avoidance (CRPA) and Conditioned reflex active avoidance (CRAA). Avoidance learning and retention were evaluated using standard paradigms. For URPI, latency to enter the dark

compartment was measured during acquisition and retention sessions; reduced retention latency indicates impaired memory consolidation. For CRPA, avoidance responses across trials were recorded as a measure of conditioned learning.

Synaptosome isolation. Brain tissue was homogenized in ice-cold sucrose buffer (0.32 M sucrose, 0.01 M Tris-HCl, 0.5 mM EDTA, pH 7.4) and subjected to differential centrifugation (4,500 rpm, 10 min; then 14,000 rpm, 20 min) to obtain a crude synaptosomal fraction.

Fluo-4AM Ca^{2+} recordings. Synaptosomes were loaded with Fluo-4AM and fluorescence was recorded over time. Measurements were performed in Ca^{2+}-containing medium (2 mM $CaCl_2$) and, where indicated, under Ca^{2+}-free conditions using EGTA. Ca^{2+} kinetics were summarized by peak amplitude ($\Delta F/F_o$), area under the curve (AUC; $\Delta F/F_o \cdot s$) and decay time constant (τ).

Oxidative stress assessment. Lipid peroxidation was quantified as MDA in blood and brain homogenates (TBARS approach), expressed as µmol per mg tissue.

Statistics. Data are presented as mean±SEM. Normality was evaluated using the Shapiro–Wilk test. Between-group comparisons were analyzed using one-way ANOVA with Dunnett's post hoc test (or Kruskal–Wallis with Dunn's correction if assumptions were violated). Statistical significance was defined as $p<0.05$.

Table 1. Experimental groups and treatment regimens.

Group	Condition / model	Treatment (dose)	Route & schedule	n
1	Control	Vehicle	Matched handling	6
2	$AlCl_3$ model	$AlCl_3$ (10 mg/kg)	i.p., once daily × 7 days	6
3	$AlCl_3$ + G-31	G-31 (50 mg/kg)	i.p., per protocol	6
4	$AlCl_3$ + G-31	G-31 (50 mg/kg)	Intranasal, per protocol	6
5	$AlCl_3$ + G-31	G-31 (50 mg/kg)	Per os, per protocol	6

RESULTS

$AlCl_3$ induces robust oxidative stress. The model significantly elevated lipid peroxidation in both systemic and brain compartments. MDA increased from 11.4±0.1 to 30.2±0.3 µmol/mg tissue in blood and from 4.54±0.4 to 8.35±0.2 µmol/mg tissue in brain homogenates ($p<0.05$–0.01).

Behavioral suppression in open field and avoidance tasks. Relative to controls, $AlCl_3$-treated rats exhibited a reduction of exploratory drive and locomotion in the open field, including decreased horizontal crossings and vertical rearing, alongside changes in emotionality-related indices. Exposure to $AlCl_3$ resulted in behavioral passivity and decreased exploratory activity: decreased locomotor activity, reduced rearing and orientation, and altered profiles of emotional reactivity markers. These results are consistent with dysfunction of the hippocampal-prefrontal networks and decreased cholinergic modulation. In the correction group receiving G-31, these results were partially restored, indicating increased efficiency of synaptic transmission (Figure 1).

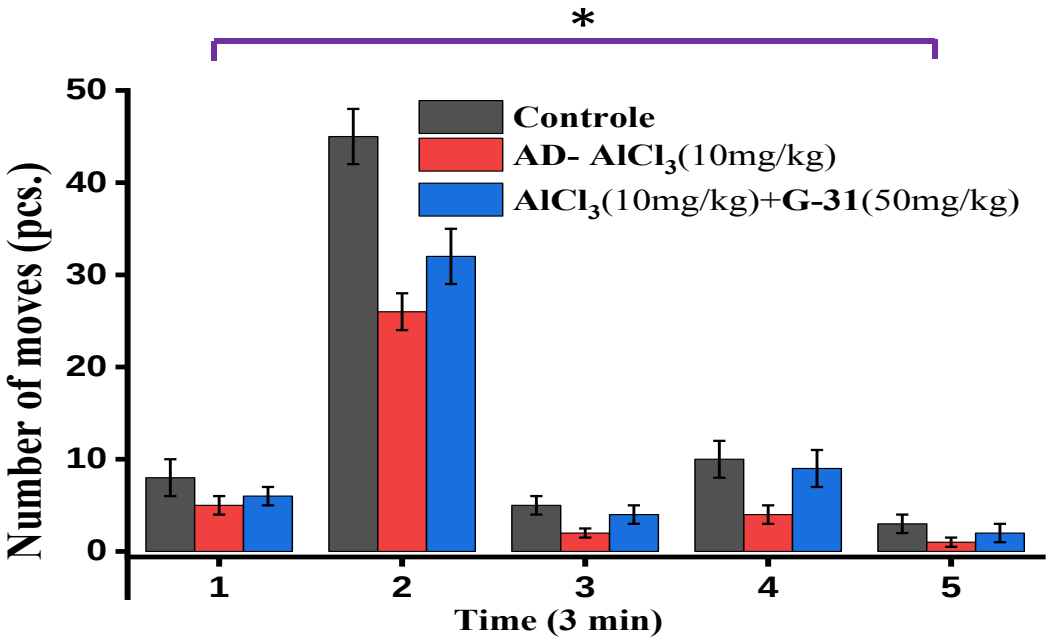

Figure 1. Main parameters of the open field test: control group, $AlCl_3$-induced AD model (10 mg/kg, i.p., 7 days), and correction with G-31 (50 mg/kg, i.p., 3 days). Data are presented as mean ± SEM (n=6). *p<0.05 compared with the control group; #p<0.05 compared with the $AlCl_3$ group.

In cognitive paradigms, the model reduced Conditioned reflex passive avoidance (CRPA) and impaired Conditioned reflex active avoidance (CRAA).

In the CRPA test, retention scores were reduced in the $AlCl_3$ model, i.e., the time to entry into the dark chamber was shortened. This was associated with dysregulation of hippocampal synaptic plasticity (LTP) and Ca^{2+}-dependent signaling, which may lead to Ca^{2+} overload and OS memory consolidation via glutamate/NMDA. In the G-31-corrected group, retention scores improved, indicating partial restoration of synaptic plasticity (Figure 2).

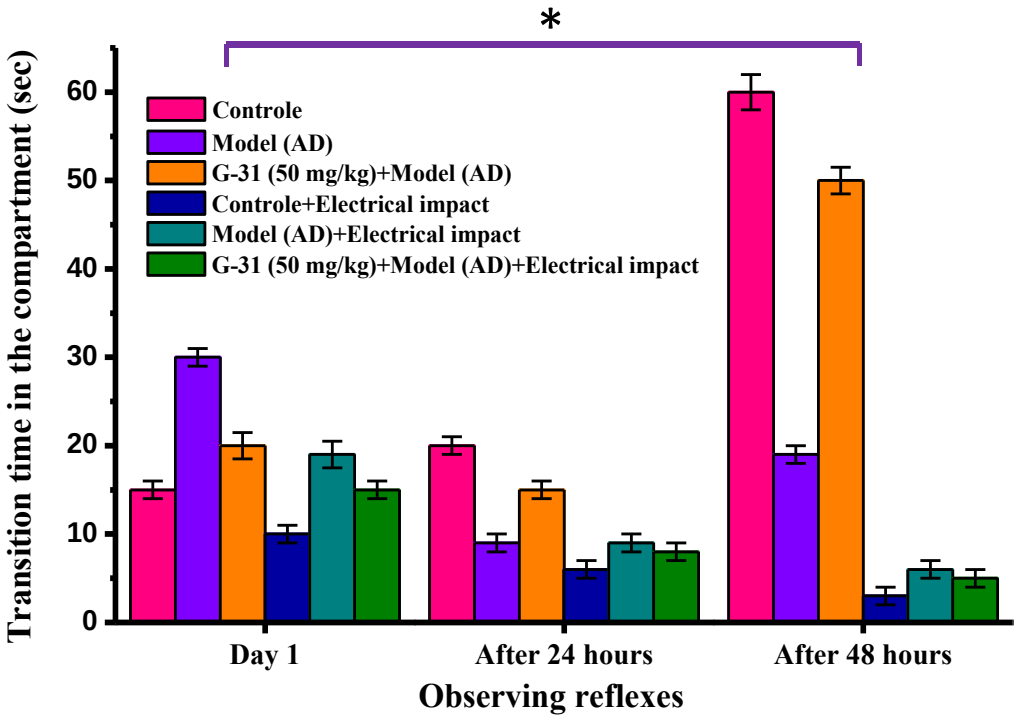

Figure 2. The conditioned passive avoidance reflex test (CRPA): time to entering the dark chamber in the control group, the $AlCl_3$ model group, and the G-31 correction group. Data are presented as mean ± SEM (n=6). *p<0.05 compared with the control group; #p<0.05 compared with the $AlCl_3$ group. µM

Synaptosomal Ca^{2+} dysregulation. Synaptosomal Fluo-4AM recordings revealed that $AlCl_3$ altered Ca^{2+} transient shape (peak/AUC and/or decay kinetics) compared with control synaptosomes, consistent with disrupted Ca^{2+} homeostasis.

In synaptosomal fluorescence analysis, Ca^{2+} content increased by an average of 52±4.5% in the control group ($p<0.05$) and 78±5.1% in the AC model group ($p<0.05$) with the addition of L-glutamate (10^{-4} M). This indicates an increase in glutamatergic excitotoxicity and a component of Ca^{2+} overload [20-24]. Changes were also observed under Ca^{2+} chelation (EGTA) conditions (Figure 3). Reduced synaptosomal Ca^{2+} response and/or accelerated clearance in G-31-corrected groups indicates restoration of the functional balance of Ca^{2+} influx pathways (receptor-gated and voltage-gated channels) and Ca^{2+} release systems (PMCA/NCX).

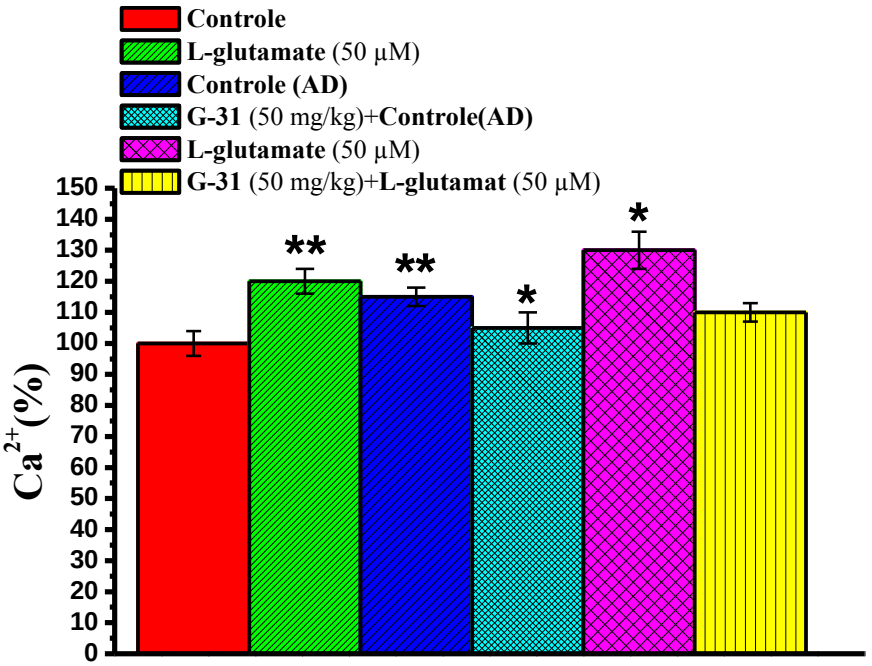

Figure 3. Changes in synaptosome Ca^{2+} levels (Fluo-4 AM) under correction with polyphenol G-31: responses in the control group, the $AlCl_3$ model group, and the G-31 group under basal and L-glutamate (10^{-4} M)/EGTA conditions. Data are presented as mean ± SEM (n=6). *p<0.05 compared with control; #p<0.05 compared with $AlCl_3$ group.

Corrective profile of polyphenol G-31. G-31 administration partially normalized behavioral and synaptosomal Ca^{2+} readouts toward the control pattern. In this dataset, intranasal delivery produced the most prominent behavioral recovery in open-field endpoints, suggesting improved CNS delivery efficiency.

DISCUSSION

This study integrates behavioral, biochemical and synaptic-level endpoints to support a mechanistic link between $AlCl_3$-induced oxidative injury and Ca^{2+}-dependent synaptic dysfunction. The increase in MDA confirms that the model generates substantial lipid peroxidation, which can compromise membrane fluidity, receptor/ion-channel function and mitochondrial bioenergetics [3,7–9]. At the systems level, reduced exploration/locomotion and weaker avoidance learning/retention are consistent with hippocampo–prefrontal network impairment and reduced plasticity efficacy.

Ca^{2+} dyshomeostasis is a well-established vulnerability factor in AD, where Ca^{2+} influx through glutamatergic pathways and reduced clearance/buffering can disrupt LTP and promote neurodegeneration [10–12]. Moreover, the distribution of NMDAR signaling between synaptic (plasticity-supporting) and extrasynaptic (stress-promoting) pools is a key determinant of whether Ca^{2+} signals drive CREB-dependent memory programs or trigger maladaptive pathways [11,26]. Therefore, synaptosomal Ca^{2+} kinetics (peak/AUC/τ) provide a functional and mechanistically interpretable readout bridging redox injury to neurotransmission.

The partial rescue observed with polyphenol G-31 is consistent with multi-target polyphenol pharmacology.

Polyphenols can reduce ROS and lipid peroxidation, chelate metals that catalyze redox cycling, and attenuate inflammatory transcription programs such as NF-κB [14–16]. Additionally, polyphenols can facilitate plasticity-associated signaling (BDNF/CREB), which is necessary for long-term memory formation [19,25]. From a biophysical standpoint, decreased membrane oxidative damage should restore receptor/ion-channel conformational flexibility and stabilize Ca^{2+} entry/clearance dynamics [27,28], thereby improving the signal-to-noise ratio of synaptic transmission.

Limitations and next steps. The dissertation dataset establishes a coherent pattern across oxidative stress, behavior and synaptosomal Ca^{2+}. To increase mechanistic resolution, follow-up experiments should pharmacologically dissect Ca^{2+} sources and clearance (e.g., depolarization-evoked Ca^{2+} entry; NMDAR antagonism; N-type channel blockade; PMCA/NCX contribution) and quantify pathway-level biomarkers (e.g., CREB phosphorylation, BDNF, and inflammatory mediators).

CONCLUSION

$AlCl_3$ exposure produced an AD-like phenotype characterized by increased lipid peroxidation, behavioral suppression in exploration and avoidance paradigms, and synaptosomal Ca^{2+} dysregulation. Polyphenol fraction G-31 provided partial correction of these endpoints, with intranasal delivery showing the most pronounced behavioral improvement in this dataset. These results support a working model in which oxidative membrane injury and Ca^{2+} dyshomeostasis cooperate to drive cognitive decline, and polyphenols counteract this process via antioxidant/metal-chelating, anti-inflammatory and Ca^{2+}-modulatory mechanisms.

Declarations

Data availability. Data supporting the findings are available from the corresponding author upon reasonable request.

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Conflict of interest. The authors declare no competing interests.

REFERENCES

1. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer’s disease. Lancet. 2021;397(10284):1577–1590. doi:10.1016/S0140-6736(20)32205-4.
2. Querfurth HW, LaFerla FM. Alzheimer’s disease. N Engl J Med. 2010;362(4):329–344. doi:10.1056/NEJMra0909142.
3. Bai R, Guo J, Ye X-Y, Xie Y, Xie T. Oxidative stress: the core pathogenesis and mechanism of Alzheimer’s disease. Ageing Res Rev. 2022;77:101619. doi:10.1016/j.arr.2022.101619.
4. Rao YL, Ganaraja B, Murlimanju BV, Joy T, Krishnamurthy A, Agrawal A. Hippocampus and its involvement in Alzheimer’s disease: a review. 3 Biotech. 2022;12:55. doi:10.1007/s13205-022-03123-4.
5. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003;463(1–3):3–33. doi:10.1016/S0014-2999(03)01272-X.
6. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40–43. doi:10.1515/intox-2017-0006.
7. Bhattacharjee S, Zhao Y, Hill JM, Percy ME, Lukiw WJ. Aluminum and its potential contribution to Alzheimer’s disease. Front Aging Neurosci. 2014;6:62. doi:10.3389/fnagi.2014.00062.
8. Zhang L, Jin C, Lu X, et al. Aluminium chloride impairs long-term memory and downregulates cAMP-PKA-CREB signalling in rats. Toxicology. 2014;323:95–108. doi:10.1016/j.tox.2014.06.011.
9. Skalny AV, Aschner M, Jiang Y, et al. Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. Adv Neurotoxicol. 2021;5:1–34. doi:10.1016/bs.ant.2020.12.001.
10. Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer’s disease. Trends Neurosci. 2008;31:454–463.
11. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci. 2002;5(5):405–414. doi:10.1038/nn835.
12. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer’s disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol. 1991;30(4):572–580. doi:10.1002/ana.410300410.
13. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science.

2016;352(6286):712–716. doi:10.1126/science.aad8373.

14. Mamun AA, Shao C, Geng P, Wang S, Xiao J. Polyphenols targeting NF-κB pathway in neurological disorders: What we know so far? Int J Biol Sci. 2024;20(4):1332–1355. doi:10.7150/ijbs.90982.

15. Lakey-Beitia J, Burillo AM, La Penna G, Hegde ML, Rao KS. Polyphenols as potential metal chelation compounds against Alzheimer's disease. J Alzheimers Dis. 2021;82(s1):S335–S357. doi:10.3233/JAD-200185.

16. Kaluza M, Ksiazek-Winiarek D, Szpakowski P, et al. Polyphenols in the central nervous system: Cellular effects and liposomal delivery approaches. Int J Mol Sci. 2025;26(13):6477. doi:10.3390/ijms26136477.

17. Singh NA, Bhardwaj V, Ravi C, et al. EGCG nanoparticles attenuate aluminum chloride induced neurobehavioral deficits, beta amyloid and tau pathology in a rat model of Alzheimer's disease. Front Aging Neurosci. 2018;10:244. doi:10.3389/fnagi.2018.00244.

18. Cheng D, Xi Y, Cao J, et al. Protective effect of apple polyphenol extract against aluminum-induced cognitive impairment and oxidative damage in rat. Neurotoxicology. 2014;45:111–120. doi:10.1016/j.neuro.2014.10.006.

19. Fiore M, Terracina S, Ferraguti G. Brain neurotrophins and plant polyphenols: A powerful connection. Molecules. 2025;30(12):2657. doi:10.3390/molecules30122657.

20. Khoshimov, N. N., Saidmurodov, S. A., & Rakhimov, R. N. (2021). The Mechanism of action of polyphenol on changes in the dynamics of calcium in the synaptosomes of the rat brain against the background of glutamate. The American journal of applied sciences, 3 (03), 48-55.

21. Mukhtorov, A. A., Mamadaminov, R. R., Khoshimov, N. N., Nasirov, K. E., Rakhimov, R. N., & Gaybullo, L. X. (2022). Regulation of transport of Ca2+ NMDA-receptors in rat brain synaptosomes under the influence of polyphenols. European Journal of Medicine, 10(1), 3-11.

22. Rakhimov, R. N., Khoshimov, N. N., Kurbanova, A. D., Komilov, K. U., Makhmanov, D. M., Kadirova, S. O., & Abdulladjanova, N. G. (2021). Isolation of new ellagitannins from plants of Euphorbiaceous and its effect on calcium transport in the nerve cell of the rat brain. Annals of the Romanian Society for Cell Biology, 25(6), 2758-2768.

23. Khoshimov, N. N., Rahimova, G. L., Mirzakulov, S. O., Azizov, V. G., Abduboqiyev, A. R., & Rakhimov, R. N. (2021). Study of the Neuroprotective Properties of Biologically Active Compounds. Annals of the Romanian Society for Cell Biology, 25(6), 2775-2782.

24. Khoshimov, N. N., & Nasirov, K. E. (2017). Action of Cytisinum on the Transport Mediators and Calcium Channel of Glutamatergic Neurotransmitter Systems of the NMDA Receptor. European Journal of Medicine, (5-2), 56-63.

25. Khoshimov, N. N., Raimova, G. M., Nasirov, K. E., Rakhimov, R. N., & Azizov, V. G. (2020). The Effect of Sp-6 On The Transport of Mediators of NMDA-Receptors and Ca 2+-channels in Synaptosomes of rat brain. European Journal of Molecular & Clinical Medicine, 7(3), 2435-2446.

26. Khoshimov, N. N., Kabil, N. E., & Eshbakova, K. A. (2015). Research influence biological active agents in the course of regulation of functional activity of platelets and system of a haemostasis. European Journal of Medicine, 2, 88-93.

27. Khoshimov, N. N., Mukhtorov, A. A., Nasirov, K. E., Rakhimov, R. N., & Mamadaminov, R. R. (2022). Effects of Polyphenols on Changes in the Transport of Ca2+ NMDA-receptors Under the Influence of L-glutamate against the Background of Alzheimer's Disease. Journal of Pharmaceutical Negative Results, 13, 1322-1332.

28. Khoshimov, N. N., Nasirov, K. E., Raimova, G. M., Musaeva, M. K., Azizov, V. G., Turaev AS, M. S., ... & Abdusalomov Sh, A. (2021). Study of the effect of polysaccharides on hemostasis. The American journal of medical sciences and pharmaceutical research, 3(01), 131-138.