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Polyphenol Modulation of Synaptosomal Ca^{2+} Signaling at NMDA Receptors

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Abstract: Background: NMDA receptor (NMDAR) overactivation and the resulting Ca^{2+} overload represent a convergent biophysical pathway implicated in synaptic failure in Alzheimer's disease (AD) [1]. Memantine is a clinically used uncompetitive NMDAR antagonist that targets the channel pore, but ligand-site modulation may offer a distinct pharmacological profile.

Methods: Rat brain synaptosomes were isolated by differential centrifugation and loaded with Fluo-4 AM to monitor cytosolic Ca^{2+} signals. NMDAR-dependent responses were evoked with glutamate (50 µM) and glycine (50 µM). The polyphenol G-41 was tested across 10–100 µM and concentration–response relationships were analyzed using the Hill equation (n = 6 independent preparations). To map the target site(s), we assessed interactions with Zn^{2+} and Mg^{2+} (5 µM; additional tests 10–100 µM in the AD condition), and compared G-41

effects with memantine (50 μM). GABAergic context was probed using GABA (100 μM), picrotoxin (50 μM), diazepam (10–100 μM), and phenobarbital (10–100 μM).

Results: G-41 produced concentration-dependent suppression of both glutamate- and glycine-driven Ca^{2+} signals. The apparent IC_{50} values were 63.9 ± 2.13 μM for the glutamate-dependent component and 42.93 ± 3.06 μM for the glycine-dependent component, with steep Hill coefficients (|nH| ≈ 3.35–6.27), consistent with multi-step receptor gating and/or integrated Ca^{2+} handling. Zn^{2+} and Mg^{2+} did not measurably alter the G-41 effect, supporting a mechanism not mediated by these allosteric regulators. G-41 did not overlap with the memantine pore site, suggesting a pharmacological profile distinct from open-channel blockade.

Conclusions: G-41 is a synaptosome-level modulator of NMDAR-dependent Ca^{2+} dynamics with evidence for dual modulation of glutamate and glycine binding sites and minimal interaction with Zn^{2+}/Mg^{2+} allosteric regulation or the memantine pore site. These properties justify follow-up electrophysiology and binding studies, as well as in vivo validation in AD-relevant models.

Keywords: Alzheimer's disease; NMDA receptor; synaptosome; Ca^{2+} dynamics; Fluo-4 AM; polyphenol; memantine; glutamate; glycine.

Introduction: Alzheimer's disease (AD) is characterized by progressive cognitive decline accompanied by synaptic dysfunction and neuronal loss. At the molecular level, soluble amyloid-β oligomers, tau pathology, neuroinflammation, and oxidative stress converge on a shared biophysical endpoint: dysregulation of Ca^{2+} homeostasis at synapses [1,2]. Excessive Ca^{2+} entry through ionotropic glutamate receptors—particularly NMDARs—can initiate mitochondrial overload, ROS generation, and impairment of synaptic plasticity [1,2]. Accordingly, NMDAR modulation remains a rational pharmacological strategy.

NMDARs are heteromeric ion channels that require concurrent binding of glutamate (typically on GluN2 subunits) and glycine/D-serine (on GluN1 subunits) to gate Ca^{2+} influx [3]. Memantine is a clinically used low-to-moderate affinity, uncompetitive antagonist that preferentially blocks excessive activation by occupying the channel pore [4-6]. However, pore-block mechanisms may not fully address ligand-site driven gating, and alternative or complementary approaches targeting glutamate and glycine sites could offer distinct efficacy–tolerability profiles.

Polyphenols constitute a large class of bioactive molecules with antioxidant, membrane-stabilizing, and receptor-modulating properties, and several polyphenols have been proposed as modulators of excitotoxic pathways relevant to neurodegeneration [7-13]. In this work, we investigated the polyphenol G-41 as a candidate modulator of synaptosomal Ca^{2+} dynamics, focusing on its interaction with glutamate and glycine binding sites, its potential relationship to Zn^{2+}/Mg^{2+} allosteric regulation, and its pharmacological separation from memantine.

METHODS

1. Animals and ethics

Adult male rats were used as the source of brain tissue for synaptosome isolation. All procedures complied with national/institutional guidelines for laboratory animal care. Ethical approval was obtained from the local ethics committee. The research protocol involving animals was approved by the Institutional Ethics Committee of the Institute of Biophysics and Biochemistry at the National University of Uzbekistan (IBB-NUU regulations dated February 17, 2026), and all procedures were conducted in accordance with relevant national and institutional guidelines for the care and use of laboratory animals.

2. Synaptosome preparation

Synaptosomes were prepared from rat brain tissue using differential centrifugation under cold conditions, as previously described [14,15]. Briefly, tissue was homogenized in 0.32 M sucrose buffer containing 0.01 M Tris-HCl and 0.5 mM EDTA (pH 7.4). The homogenate was centrifuged (1300 rpm, 10 min) to remove nuclei and debris; the supernatant was centrifuged (12600 rpm, 15 min) to obtain the crude synaptosomal pellet. The pellet was resuspended and washed by a further centrifugation step (10000 rpm, 15 min), and the final synaptosome suspension was prepared in the working buffer for fluorescence assays.

3. Fluo-4 AM Ca^{2+} fluorescence assay

Synaptosome suspensions were loaded with Fluo-4 AM and incubated for 15 min before measurements. Fluorescence intensity was recorded under standard conditions. Ca^{2+} responses were evoked by adding agonists/modulators as described below. The primary endpoints were the peak response and integrated response (AUC) of Fluo-4 fluorescence.

4. Pharmacological design

To evoke NMDAR-dependent Ca^{2+} responses, glutamate (50 µM) and glycine (50 µM) were applied. G-41 polyphenol was tested in the range 10–100 µM. To probe allosteric modulation, Zn^{2+} and Mg^{2+} were applied at 5 µM in the baseline panel; additional 10–100 µM tests were performed in the AD condition. Memantine (50 µM) served as a reference pore-site antagonist [16]. To evaluate crosstalk with inhibitory signaling, GABA (100 µM), picrotoxin (50 µM), diazepam (10–100 µM), and phenobarbital (10–100 µM) were used.

5. Statistics

Data are presented as mean ± SEM. Normality was assessed (Shapiro–Wilk test). Concentration–response parameters were estimated using the Hill equation [16]. For multiple comparisons versus control, one-way ANOVA with Dunnett's post-hoc test was applied; if normality was violated, Kruskal–Wallis with Dunn's correction was used. A p value < 0.05 was considered statistically significant.

RESULTS

1. G-41 inhibits glutamate- and glycine-dependent Ca^{2+} signaling in synaptosomes

G-41 (10–100 µM) reduced Fluo-4 AM fluorescence responses elicited by glutamate (50 µM) and glycine (50 µM) in a concentration-dependent manner. Hill analysis yielded IC_{50} = 63.9 ± 2.13 µM for the glutamate-dependent component and IC_{50} = 42.93 ± 3.06 µM for the glycine-dependent component (n = 6). The steep concentration dependence (|nH| ≈ 3.35–6.27) suggests that the measured signal reflects integrated receptor gating and downstream Ca^{2+} handling rather than a simple one-step inhibition.

2. Zn^{2+} and Mg^{2+} do not alter the G-41 effect

When Zn^{2+} or Mg^{2+} (5 µM; additional 10–100 µM in AD condition) were included, no appreciable change in the G-41 inhibitory profile was observed, consistent with the absence of a major interaction at Zn^{2+}/Mg^{2+}-sensitive allosteric sites under the present conditions [3].

3. G-41 does not overlap with the memantine pore site

In comparative experiments, G-41 (50 µM) produced only a minimal effect relative to memantine (50 µM), supporting the interpretation that G-41 does not target the same open-channel pore site as memantine [4]. This pharmacological separation is consistent with ligand-site modulation.

4. Modulation in a GABAergic pharmacological context

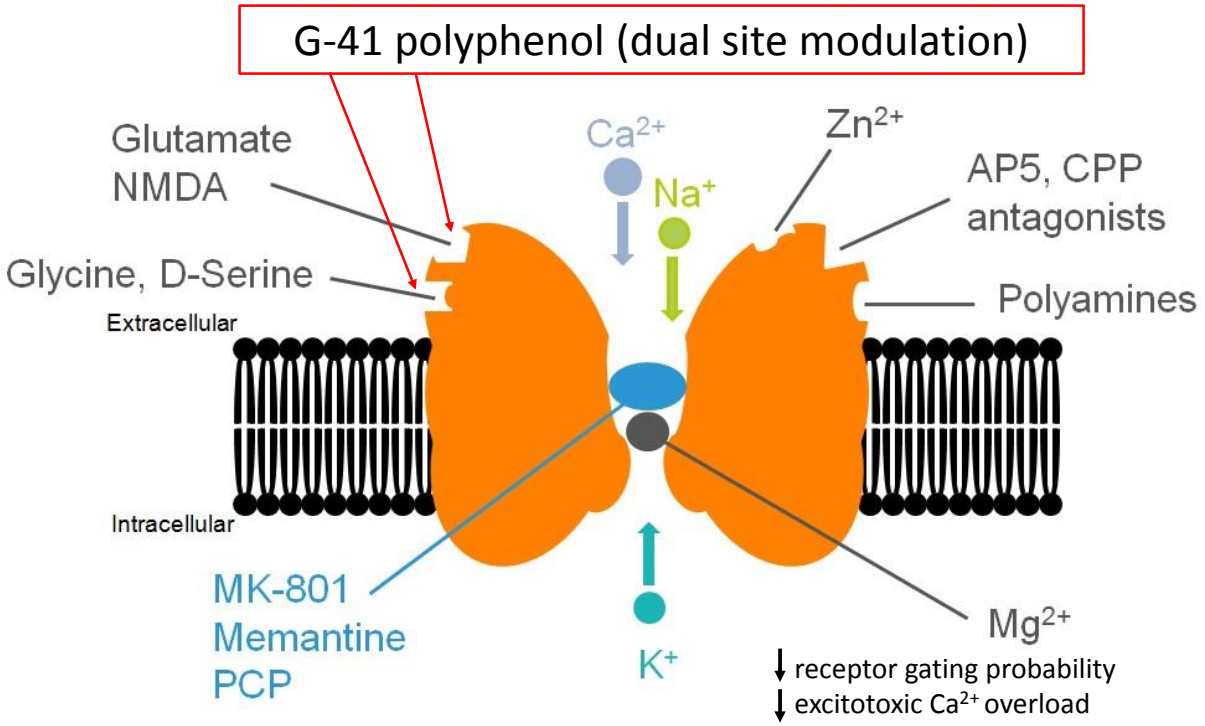
In a functional screen summarized in Table 1, G-41 (50 µM) reduced Ca^{2+} signals under glutamate or glycine stimulation (to 86 ± 2% and 83 ± 1% of control, respectively), while the presence of Zn^{2+} or Mg^{2+} produced values near control (98 ± 2% and 99 ± 1%, respectively). Inhibitory pathway modulation showed a larger dynamic range: GABA reduced the signal (76 ± 2%), diazepam and phenobarbital further suppressed responses (56 ± 2% and 59 ± 1%), whereas picrotoxin was associated with an increased signal (120 ± 1%).

Table 1. Summary of G-41 (50 µM) effects on synaptosomal Ca^{2+} fluorescence under different modulators (n = 6).

Condition (agonist/modulator)	Fluorescence (% of control, mean ± SEM)
Glutamate (50 µM)	86 ± 2
Glycine (50 µM)	83 ± 1
Zn^{2+} (5 µM)	98 ± 2
Mg^{2+} (5 µM)	99 ± 1
GABA (100 µM)	76 ± 2
Picrotoxin (50 µM)	120 ± 1
Diazepam (10–100 µM)	56 ± 2
Phenobarbital (10–100 µM)	59 ± 1

Figure 1. Proposed biophysical mechanism of G-41 action on synaptosomal Ca^{2+} dynamics.

Proposed mechanism – G-41 targets NMDA-receptor co-agonist sites and normalizes synaptosomal Ca^{2+} dynamics



NMDA-receptor co-agonist sites and normalized Ca2+ dynamics

G-41 is proposed to modulate NMDAR function primarily via interactions with glutamate and glycine binding sites, reducing receptor gating probability and limiting Ca^{2+} influx [3]. The lack of overlap with the memantine pore site supports a ligand-site mechanism distinct from open-channel blockade [4]. Reduced Ca^{2+} entry is expected to attenuate downstream excitotoxic pathways (mitochondrial overload, ROS generation) and favor recovery of synaptic function [1,2].

DISCUSSION

These synaptosome-level findings indicate that G-41 suppresses NMDAR-linked Ca^{2+} signals elicited by both glutamate and glycine. Because NMDAR activation requires co-agonist occupancy—glutamate at GluN2 and glycine/D-serine at GluN1 [3]—dual suppression supports either (i) direct binding/modulation at both sites or (ii) a dominant effect on a shared gating step that reduces contributions of both co-agonists. In either case, reducing NMDAR-mediated Ca^{2+} entry is mechanistically aligned with neuroprotection in excitotoxic AD-relevant settings [1,2].

A key mechanistic distinction is supported by the lack of measurable Zn^{2+}/Mg^{2+} interaction and the non-overlap with the memantine pore site. Zn^{2+} can inhibit certain NMDAR subtypes through high-affinity allosteric sites, whereas Mg^{2+} produces a voltage-dependent channel block [3]. The present findings suggest that G-41 does not primarily act through these pathways, reinforcing ligand-site modulation. Similarly, memantine's clinical mechanism is tied to uncompetitive pore blockade during excessive activation [2]. Pharmacological separation from this site may translate into a different activity–tolerability balance.

The steep Hill coefficients observed for inhibition likely reflect the multi-component nature of the experimental endpoint. In synaptosomes, Fluo-4 fluorescence integrates receptor gating, local membrane excitability, Ca^{2+} entry routes, buffering, and extrusion via Ca^{2+} pumps and exchangers. Oxidative stress can impair PMCA function in brain membranes [17] and alter NCX mobility and function in neurons [18-22], both of which would reshape Ca^{2+} peak, AUC, and clearance kinetics. Polyphenols can stabilize redox balance and membrane integrity and may therefore influence Ca^{2+} handling alongside receptor-site actions [7,8]. Hence, a plausible integrated mechanism for G-41 is partial ligand-site antagonism combined with restoration of redox-sensitive Ca^{2+} homeostasis components.

The broader pharmacological screen in a GABAergic context suggests that synaptosomal Ca^{2+} signals depend on the balance between excitatory and inhibitory influences even in reduced preparations. The reduction of Ca^{2+} signals under diazepam/phenobarbital conditions is consistent with

enhanced inhibitory tone and reduced depolarization-coupled Ca^{2+} entry, whereas the increased signal with picrotoxin is consistent with disinhibition effects.

Limitations: The current dataset is ex vivo. While synaptosomes provide a controlled model of presynaptic function, they cannot fully recapitulate intact neuronal network behavior. Future work should combine receptor binding assays, patch-clamp electrophysiology, and oxidative stress/mitochondrial readouts to establish a causal chain from receptor engagement to Ca^{2+} phenotype. In vivo validation in AD-relevant models and behavioral phenotyping would be required to establish translational potential.

CONCLUSIONS

G-41 polyphenol suppresses synaptosomal Ca^{2+} signals driven by glutamate and glycine, with apparent IC_{50} values in the 40–65 µM range and a pharmacological profile distinct from Zn^{2+}/Mg^{2+} allosteric regulation and the memantine pore site. These findings support G-41 as a candidate ligand-site modulator of NMDAR-dependent Ca^{2+} dysregulation, motivating deeper mechanistic studies and in vivo validation.

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Conflict of interest. The authors declare no competing interests.

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