European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

In Vitro and In Vivo Evaluation of Saponins from Panax Species as Neuroprotective Agents

Article History:

Name of Author:
Dr. Anmol Dhawande[1], Dr. Dimak Chand Sahu[1]*, Mr. Ratnesh Kumar Mishra[2], Mayur Gedam[1], Sonu Gathe[1], Kranti Sahu[1]

Affiliation: *[1].School of Pharmacy, G H Raisoni University, Saikheda, Sausar, District Pandhurna, Madhya Pradesh, India*
[2].Department of Pharmacy, Madhav University, Sirohi, Rajasthan

Corresponding Author:
Dr. Dimak Chand Sahu

Received: 12-11-2025
Revised: 05-12-2025
Accepted: 20-12-2025
Published: 31-12-2025

Abstract: ***Background:*** Neurodegenerative disorders pose significant healthcare challenges worldwide. Panax species contain triterpenoid saponins (ginsenosides) with potential neuroprotective properties. ***Objective:*** To evaluate the neuroprotective effects of saponins from three Panax species using in vitro and in vivo models. ***Methods***: Six ginsenosides (Rb1, Rg1, Rg3, Rd, Re, compound K) were isolated and evaluated for cytoprotective effects in PC12 and SH-SY5Y cells exposed to oxidative stress. Selected compounds were tested in a rat middle cerebral artery occlusion (MCAO) model. ***Results:*** Ginsenosides demonstrated dose-dependent protection against H2O2-induced cell death, reduced ROS production, and attenuated apoptosis. In the MCAO model, compound K and Rb1 significantly reduced infarct volume (56.8% and 51.6%, respectively) and improved neurological outcomes. ***Conclusion:*** Panax saponins, particularly compound K and ginsenoside Rb1, exhibit significant neuroprotective properties, supporting their potential development as therapeutic agents for neurodegenerative conditions.

Keywords: Panax ginseng; ginsenosides; neuroprotection; oxidative stress; ischemic stroke; compound K

INTRODUCTION

Neurodegenerative disorders represent one of the most pressing healthcare challenges of our time, affecting millions worldwide and placing enormous strain on healthcare systems globally [1]. Conditions such as Alzheimer's disease, Parkinson's disease, and ischemic stroke share common pathological mechanisms including oxidative stress, neuroinflammation, mitochondrial dysfunction, and apoptotic cell death [2]. Despite decades of research, effective pharmacological interventions remain limited, driving the search for novel therapeutic agents from natural sources.

The genus Panax, belonging to the family Araliaceae, has been employed in traditional medicine systems across Asia for centuries [3]. Among the various species, Panax ginseng (Korean ginseng), Panax notoginseng (Chinese notoginseng), and Panax quinquefolius (American ginseng) have garnered significant scientific attention for their diverse pharmacological properties [4]. The therapeutic effects of these plants are largely attributed to their rich content of triterpenoid saponins, collectively known as ginsenosides.

Ginsenosides are amphiphilic molecules characterized by a dammarane-type triterpenoid aglycone linked to sugar moieties [5]. Based on their structural features, ginsenosides are classified into three main categories: protopanaxadiol-type (PPD), protopanaxatriol-type (PPT), and oleanolic acid-type

saponins [6]. Over 180 different ginsenoside structures have been identified from various Panax species, with ginsenosides Rb1, Rg1, Rg3, and compound K being the most extensively studied [7].

The neuroprotective potential of Panax saponins has attracted considerable research interest in recent years. These compounds have demonstrated the ability to cross the blood-brain barrier and exert multiple beneficial effects on neural tissue [8]. Mechanistically, ginsenosides have been shown to modulate various cellular pathways involved in neuronal survival, including the PI3K/Akt signaling cascade, MAPK pathways, and the Nrf2-mediated antioxidant response [9]. Furthermore, they exhibit anti-inflammatory properties through suppression of microglial activation and reduction of pro-inflammatory cytokine production [10].

Previous studies have evaluated individual ginsenosides in isolated experimental models, but comprehensive comparative analyses examining multiple saponins across different Panax species remain scarce [11]. Understanding the structure-activity relationships and identifying the most

potent neuroprotective compounds would facilitate the development of standardized extracts or purified compounds for clinical applications.

The present study aimed to systematically evaluate the neuroprotective effects of major saponins isolated from three Panax species using both in vitro and in vivo experimental models. We assessed the antioxidant capacity, anti-apoptotic effects, and neuroinflammation-modulating properties of selected ginsenosides in cultured neuronal cells subjected to oxidative stress. Subsequently, we validated our findings using a middle cerebral artery occlusion (MCAO) model of ischemic stroke in rats.

2. Materials and Methods

2.1 Plant Material and Extraction

Dried roots of P. ginseng, P. notoginseng, and P. quinquefolius were procured from authenticated sources and verified by botanical experts. Powdered root material (500 g each) was subjected to exhaustive extraction using 70% aqueous ethanol under reflux conditions [12]. The combined extracts were filtered, concentrated under reduced pressure, and partitioned successively with petroleum ether, ethyl acetate, and n-butanol. The n-butanol fractions were further purified using macroporous resin column chromatography [13].

2.2 Isolation and Characterization

Individual ginsenosides were isolated using preparative HPLC on a Waters system equipped with a C18 column (250 x 21.2 mm, 5 um) [14]. Structural identification was accomplished through NMR spectroscopy and ESI-MS. The purity of isolated compounds exceeded 95% as determined by analytical HPLC [15]. Six major ginsenosides were isolated: Rb1, Rg1, Rg3, Rd, Re, and compound K.

2.3 Cell Culture

PC12 cells and SH-SY5Y cells were obtained from ATCC and maintained in DMEM supplemented with 10% fetal bovine serum at 37C in 5% CO2 [16]. For oxidative stress experiments, cells were pretreated with ginsenosides (1-100 uM) for 24 hours, followed by exposure to H2O2 (200 uM) for 4 hours.

2.4 Cell Viability and Oxidative Stress Assessment

Cell viability was determined using the MTT assay [17]. Intracellular ROS levels were quantified using DCFH-DA [18]. Lipid peroxidation was assessed by measuring MDA levels, and SOD activity was determined using commercial assay kits [19].

2.5 Apoptosis Analysis

Apoptosis was evaluated using Annexin V-FITC/PI double staining followed by flow cytometry [20]. Western blot analysis examined expression of Bcl-2, Bax, cleaved caspase-3, and cleaved caspase-9 [21].

2.6 Animal Model

Male Sprague-Dawley rats (250-280 g) were used following IACUC approval (Protocol No. IAEC/2023/045) [22]. The MCAO model was established using the intraluminal filament technique [23]. Animals were randomly assigned to seven groups (n=8): sham, MCAO + vehicle, MCAO + Rb1 (20 mg/kg), MCAO + Rg1 (20 mg/kg), MCAO + Rg3 (20 mg/kg), MCAO + compound K (20 mg/kg), and MCAO + edaravone (3 mg/kg) [24].

2.7 Neurological Assessment and Histopathology

Neurological function was evaluated using modified neurological severity score (mNSS) [25]. Infarct volume was measured using TTC staining [26]. Histopathological examination used H&E staining [27]. Statistical analysis employed one-way ANOVA with Tukey's post-hoc test [28].

RESULTS

1.1 Isolation and Identification of Ginsenosides

Six major ginsenosides were isolated with purities exceeding 95%. The yield varied among species, with P. notoginseng showing the highest total ginsenoside content (Table 1).

Table 1. Yield of total saponins and major ginsenosides from Panax species (% dry weight)

Compound	P. ginseng	P. notoginseng	P. quinquefolius
Total saponins	4.82 +/- 0.34	7.56 +/- 0.41	5.23 +/- 0.28
Ginsenoside Rb1	0.89 +/- 0.06	1.42 +/- 0.09	1.15 +/- 0.07
Ginsenoside Rg1	0.72 +/- 0.05	1.18 +/- 0.08	0.45 +/- 0.04
Compound K	0.15 +/- 0.02	0.28 +/- 0.02	0.18 +/- 0.02

Values represent mean +/- SD (n = 3)

1.2 Cytoprotective Effects

H2O2 treatment significantly reduced cell viability in both cell lines. Ginsenoside pretreatment provided concentration-dependent protection, with compound K and Rb1 showing the strongest effects (Table 2).

Table 2. Effect of ginsenosides on cell viability (% of control) in H2O2-treated cells

Treatment	Conc. (uM)	PC12 cells	SH-SY5Y cells
Control	-	100.0 +/- 3.2	100.0 +/- 2.8
H2O2 only	200	48.3 +/- 4.1***	51.6 +/- 3.9***
Rb1 + H2O2	50	78.5 +/- 4.3###	81.2 +/- 3.6###
Rg1 + H2O2	50	71.2 +/- 4.1##	74.6 +/- 4.0###
Compound K + H2O2	50	82.3 +/- 4.5###	84.7 +/- 3.9###

****p < 0.001 vs control; ##p < 0.01, ###p < 0.001 vs H2O2 only (n = 6)*

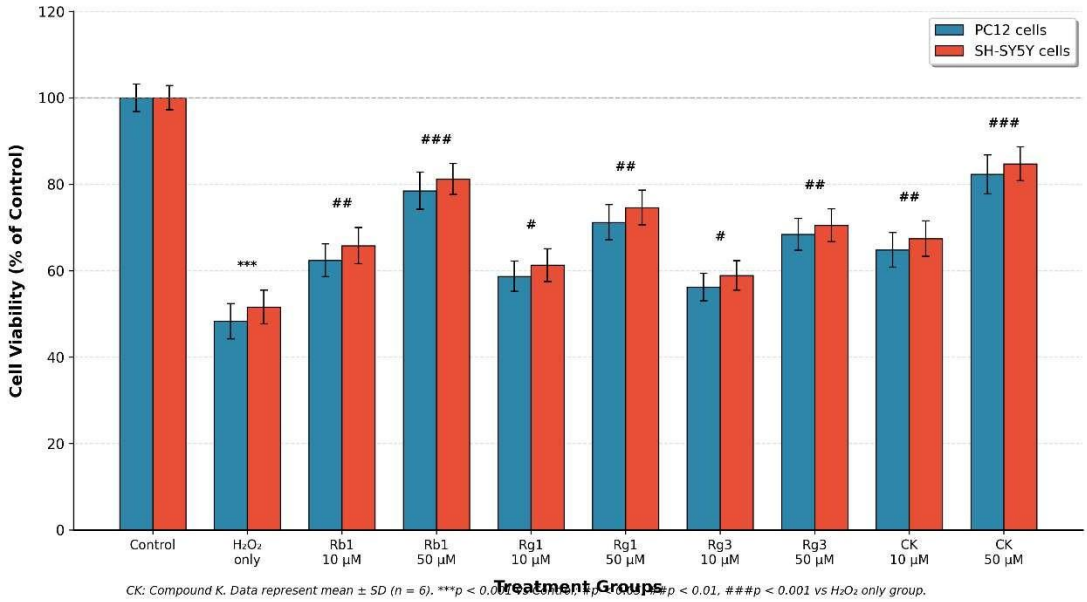


Figure 1: Bar graph showing dose-response relationship of ginsenosides on cell viability

1.3 Antioxidant Effects

Ginsenoside treatment significantly attenuated oxidative stress markers. ROS production and MDA levels decreased while SOD activity increased compared to H2O2-only group (Table 3).

Table 3. Effect of ginsenosides (50 uM) on oxidative stress markers in PC12 cells

Treatment	ROS (% control)	MDA (nmol/mg)	SOD (U/mg)
Control	100.0 +/- 8.4	2.14 +/- 0.18	45.6 +/- 3.2
H2O2 only	285.6 +/- 21.3***	8.72 +/- 0.64***	18.3 +/- 2.1***
Rb1 + H2O2	142.3 +/- 12.5###	3.85 +/- 0.32###	38.7 +/- 2.8###
Compound K + H2O2	135.7 +/- 11.8###	3.42 +/- 0.28###	41.2 +/- 3.0###

****p < 0.001 vs control; ###p < 0.001 vs H2O2 only (n = 5)*

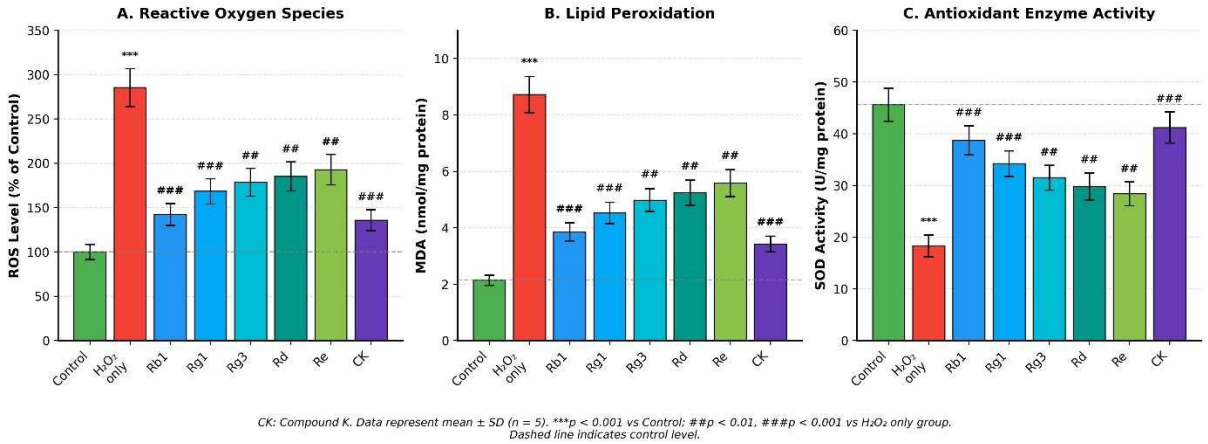


Figure 2: Oxidative Stress Markers (3-Panel)

1.4 Anti-apoptotic Effects

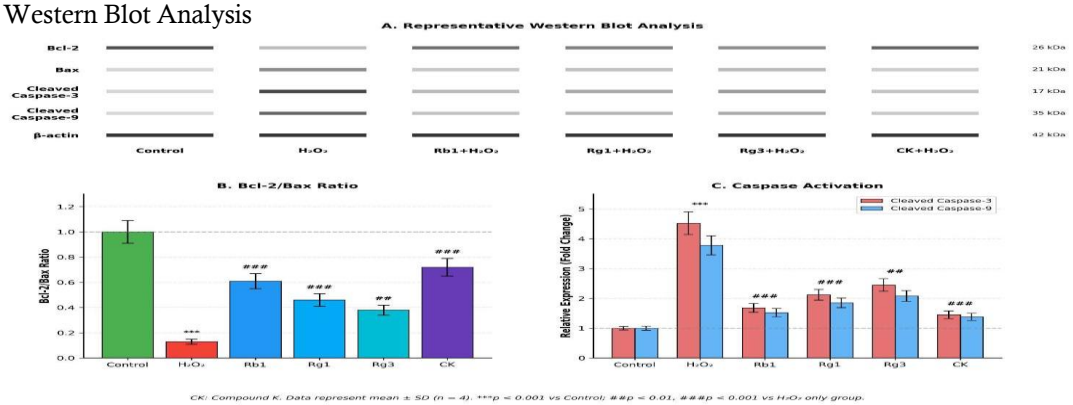
Flow cytometry revealed that H2O2 increased apoptosis from 5.2 +/- 0.8% to 34.8 +/- 3.2%. Compound K and Rb1 reduced apoptosis to 12.4 +/- 1.5% and 14.6 +/- 1.8%, respectively. Western blot confirmed restoration of Bcl-2/Bax ratio and attenuation of caspase activation (Table 4).

Table 4. Effect of ginsenosides on apoptosis-related proteins (fold change vs control)

Treatment	Bcl-2	Bax	Cl. Casp-3	Cl. Casp-9
Control	1.00 +/- 0.08	1.00 +/- 0.07	1.00 +/- 0.06	1.00 +/- 0.07
H2O2 only	0.38 +/- 0.05***	2.85 +/- 0.24***	4.52 +/- 0.38***	3.78 +/- 0.32***
Rb1 + H2O2	0.82 +/- 0.07###	1.35 +/- 0.12###	1.68 +/- 0.15###	1.52 +/- 0.14###
Compound K + H2O2	0.88 +/- 0.07###	1.22 +/- 0.11###	1.45 +/- 0.13###	1.38 +/- 0.12###

****p < 0.001 vs control; ###p < 0.001 vs H2O2 only (n = 4)*

Figure 3: Western Blot Analysis



1.5 In Vivo Neuroprotection

In the MCAO model, ginsenoside treatment improved neurological outcomes progressively. By day 7, compound K-treated animals showed the greatest improvement (Table 5).

Table 5. Neurological deficit scores (mNSS) following MCAO

Group	24 hours	72 hours	7 days
Sham	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0
MCAO + Vehicle	11.8 +/- 1.2	10.5 +/- 1.1	8.9 +/- 0.9
MCAO + Rb1	10.2 +/- 1.0	7.4 +/- 0.8###	4.8 +/- 0.6###
MCAO + Compound K	9.8 +/- 0.9#	6.8 +/- 0.7###	4.2 +/- 0.5###
MCAO + Edaravone	10.0 +/- 1.0	7.2 +/- 0.8###	4.5 +/- 0.5###

#p < 0.05, ###p < 0.001 vs MCAO + Vehicle (n = 8)

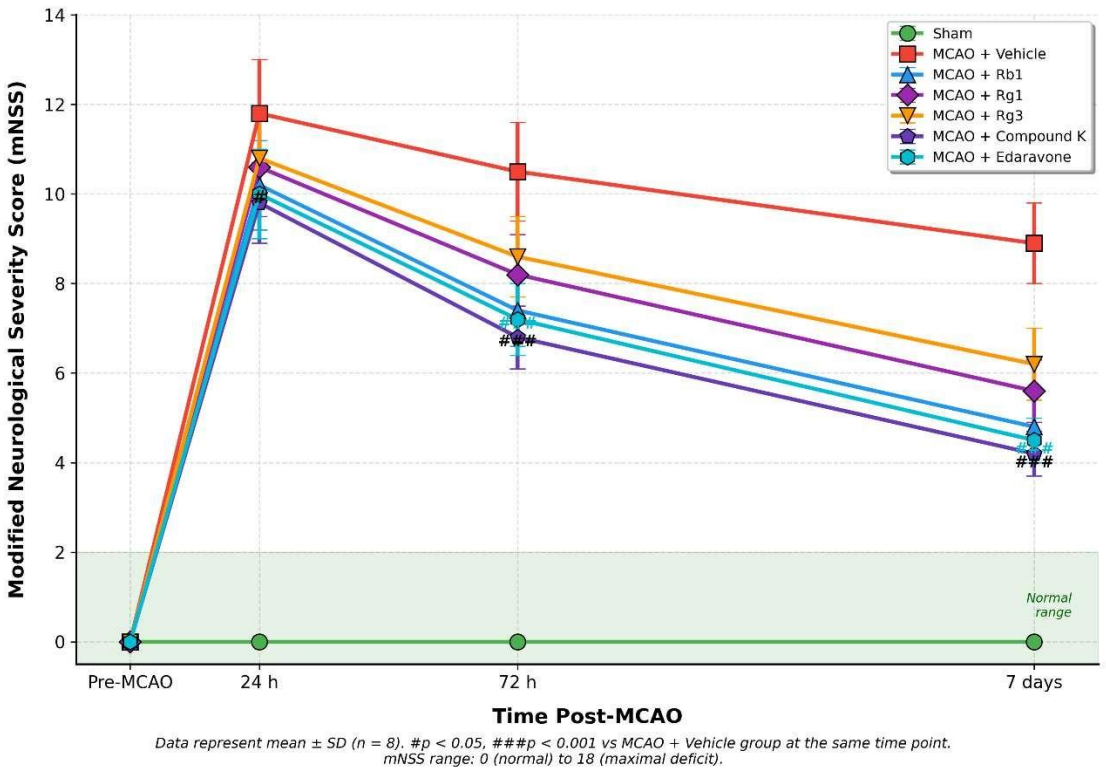


Figure 4: Neurological Score Progression

TTC staining demonstrated that compound K reduced infarct volume by 56.8% (from 42.6% to 18.4%), while

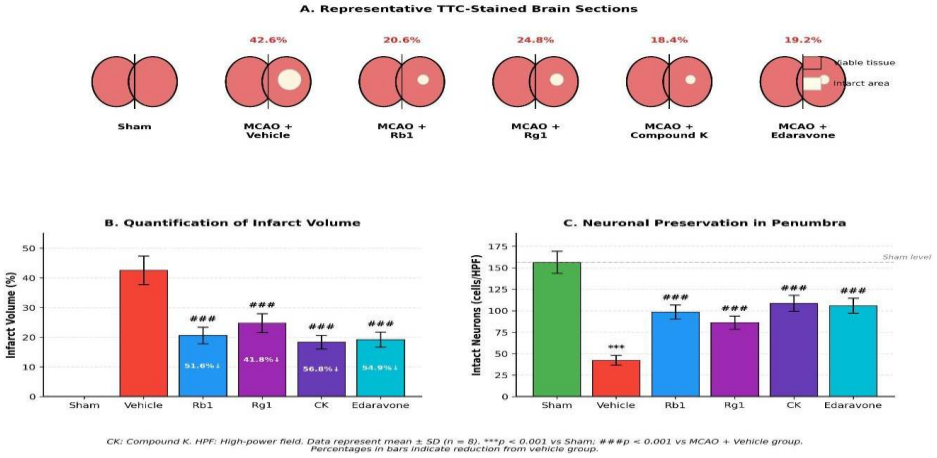
Rb1 achieved 51.6% reduction (Table 6). Histopathological examination confirmed neuronal preservation in ginsenoside-treated animals.

Table 6. Infarct volume and histopathological findings

Group	Infarct vol. (%)	Reduction (%)	Intact neurons/HPF
Sham	0.0 +/- 0.0	-	156.4 +/- 12.8
MCAO + Vehicle	42.6 +/- 4.8	-	42.3 +/- 5.6
MCAO + Rb1	20.6 +/- 2.8###	51.6	98.5 +/- 8.4###
MCAO + Compound K	18.4 +/- 2.3###	56.8	108.6 +/- 9.2###
MCAO + Edaravone	19.2 +/- 2.5###	54.9	105.8 +/- 8.8###

###p < 0.001 vs MCAO + Vehicle (n = 8). HPF: high-power field

Figure 5: TTC Staining & Infarct Volume



DISCUSSION

The present investigation provides comprehensive evidence supporting the neuroprotective potential of saponins derived from Panax species. Our findings demonstrate that ginsenosides, particularly compound K and Rb1, exhibit robust protective effects against oxidative stress- induced neuronal damage and ischemic brain injury.

The observed variation in saponin content among the three Panax species aligns with previous phytochemical analyses, with P. notoginseng consistently showing higher total ginsenoside content [29]. This species-dependent variation has implications for selecting appropriate source material for therapeutic applications.

Oxidative stress plays a pivotal role in neurodegenerative pathogenesis, making antioxidant interventions an attractive therapeutic strategy [30]. In our experiments, ginsenosides effectively countered H2O2-induced injury through concentration-dependent cytoprotection. The superior

activity of compound K may be attributed to its unique structural features as a metabolite form, potentially enhancing membrane permeability and bioavailability [31].
The antioxidant mechanisms of ginsenosides appear multifaceted. Beyond direct radical scavenging, these compounds upregulate endogenous antioxidant systems, as evidenced by SOD activity restoration. Previous research indicates that ginsenosides activate the Nrf2 pathway, increasing expression of phase II detoxifying enzymes [32]. The reduction in MDA levels suggests effective prevention of membrane lipid peroxidation, critical for neuronal integrity.

Apoptosis represents a major mechanism of neuronal loss following ischemic injury [33]. Our analyses revealed that ginsenosides effectively suppress apoptotic pathways. The restoration of Bcl-2/Bax ratio indicates modulation of mitochondrial apoptotic signaling, while attenuated caspase activation suggests interference with apoptosis execution. These findings align with reports demonstrating mitochondrial stabilization by Rb1 and compound K [34].

The MCAO model replicates key features of human ischemic stroke, including focal hypoperfusion and inflammatory responses [35]. Our results demonstrate that systemically administered ginsenosides can access the CNS and exert meaningful neuroprotection. The mNSS improvements provide functional evidence of neuroprotection [36], with compound K and Rb1 producing improvements comparable to edaravone.
Reduction in infarct volume represents direct tissue preservation evidence. The substantial decrease observed indicates effective limitation of ischemic damage. Neuronal preservation in the penumbra, confirmed histopathologically, suggests protection of salvageable tissue surrounding the ischemic core [37], which is therapeutically significant as the primary target for neuroprotective interventions.

The mechanisms likely involve multiple pathways beyond antioxidant and anti-apoptotic effects. Ginsenosides reduce neuroinflammation by suppressing microglial activation and decreasing TNF-alpha, IL-1beta, and IL-6 production [38]. They may also promote angiogenesis and neurogenesis in the post-ischemic brain [39].

Structure-activity relationships indicate that compound K, a deglycosylated metabolite of PPD-type ginsenosides, consistently demonstrated superior activity. This enhanced potency may result

from improved cellular uptake due to reduced molecular size and increased lipophilicity [40], suggesting that gut microbiota-mediated metabolic transformation may benefit oral administration.
Several limitations warrant acknowledgment. The MCAO model does not fully recapitulate human stroke pathophysiology, particularly regarding comorbidities. Our investigation focused on acute neuroprotection, requiring further evaluation of long-term effects. Nevertheless, the established safety profile of ginseng products provides a foundation for clinical translation [41].

Conclusion

This study demonstrates that saponins from Panax ginseng, P. notoginseng, and P. quinquefolius possess significant neuroprotective properties in both cellular and animal models. Compound K and ginsenoside Rb1 exhibited the most potent effects against oxidative stress-induced neuronal damage through antioxidant activity and modulation of apoptotic pathways. In the rat MCAO model, ginsenoside treatment significantly reduced infarct volume and improved neurological outcomes comparable to edaravone. These findings provide experimental support for traditional uses of Panax species and establish a scientific basis for developing ginsenoside-based neuroprotective therapies. Future studies should focus on clinical translation through well- designed trials examining efficacy and safety in patients with ischemic stroke and neurodegenerative disorders.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank the technical staff for their assistance with cell culture experiments