**Pharmacogn.** Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

# Effect and Mechanism of Ginsenoside Rg1 on Synaptic Plasticity of Oxygen-Glucose Deprivation/Reoxygenation-Induced Neuronal Injury

**Chen Yan[1,2], Yan Ma[3], Lin Li[1,4], Meng Zhang[5], Junhua Zhan[1,4], Xiang Fan[1,4]**

[1]Department of Pharmacology And Toxicology, Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, [2]Department of Traditional Chinese Medicine, Tianjin Vocational College of Bioengineering, [3]Department of Acupuncture, Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, [4]Department of Clinical Research Evaluation, Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, [5]Department of Traditional Chinese Medicine, School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, China

Submitted: 13-Dec-2019 Revised: 27-Feb-2020 Accepted: 21-Apr-2020 Published: 20-Oct-2020

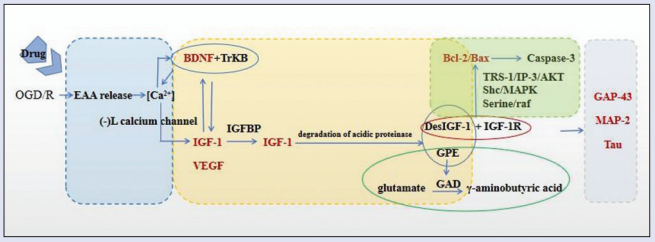
## ABSTRACT

**Background:** Ginsenoside Rg1 is the primary bioactive component of ginseng, which is a famous traditional Chinese medicine used to treat ischemic cardiovascular and cerebrovascular diseases. It has demonstrated considerable protective effects in neurons injured by ischemia/reperfusion both in *in vitro* and *in vivo* conditions. However, the effect and mechanism of action of ginsenoside Rg1 on the neural synaptic plasticity injured by ischemia/reperfusion have not yet been clarified. **Objective:** In this study, we aim to establish the model of oxygen–glucose deprivation/reoxygenation (OGD/R)-injured primary cortical neurons to mimic ischemia/reperfusion injury and investigate the mechanisms of action of ginsenoside Rg1 on the neural synaptic plasticity. **Materials and Methods:** Protective effects of ginsenoside Rg1 on neurons after OGD/R injury were measured by cell counting kit-8, lactate dehydrogenase, and apoptosis assay. The mRNA expression and activity of growth associated protein (GAP)-43, microtubule-associated protein (MAP)-2, Tau, insulin like growth factors (IGF)-1, brain derived neurotrophic factor (BDNF), and vascular endothelial growth facto r (VEGF) in neurons after OGD/R injury were measured by real-time polymerase chain reaction (RT-PCR) or enzyme-linked immunosorbent assay. The expression of apoptosis-related genes and cytosolic $Ca^{2+}$ levels in neurons were determined via RT-PCR or Rhod-2 fluorescence staining. **Results:** According to our results, ginsenoside Rg1 protected the neurons and promoted axonal regeneration and neuronal remodeling after OGD/R injury; increased the expression of GAP-43, MAP-2, Tau, IGF-1, BDNF, VEGF, and Bcl-2 and inhibited the expression of Bax; and decreased the intracellular $Ca^{2+}$ overload in OGD/R-injured neurons. **Conclusion:** Ginsenoside Rg1 can promote axonal regeneration and neuronal remodeling after OGD/R injury by upregulating the expression of synaptic remodeling proteins and endogenous neurotrophic factors, inhibiting intracellular $Ca^{2+}$ overload and regulating the expression of apoptotic genes.

**Key words:** Ginsenoside Rg1, neuron, neuroprotection, oxygen–glucose deprivation/reoxygenation, synaptic plasticity

## SUMMARY

- Ginsenoside Rg1 promoted axonal regeneration and neuronal remodeling after oxygen-glucose deprivation/reoxygenation (OGD/R) injury *in vitro*
- Ginsenoside Rg1 upregulated the expression of synaptic remodeling proteins in neuron after OGD/R injury, such as growth associated protein-43, microtubule-associated protein-2, and Tau
- Ginsenoside Rg1 upregulated the expression of endogenous neurotrophic factors in neuron after OGD/R injury, such as insulin like growth factors-1, brain derived neurotrophic factor, and vascular endothelial growth factor.




**Abbreviations used:** Akt: AKT serine/threonine kinase; BDNF: Brain derived neurotrophic factor; CCK-8: Cell counting kit-8; CNS: Central nervous system; ERK1/2: Extracellular regulated protein kinases 1/2; GAP-43: Growth associated protein-43; IGF-1: Insulin like growth factors 1; MAP-2: Microtubule-associated protein 2; NF-κB: Nuclear factor kappa-B; nNOS: Neuronal nitric oxide synthase; NO: Nitric oxide; OGD/R: Oxygen-glucose deprivation/reoxygenation; PPARγ: Peroxisome proliferator activated receptor gamma; VEGF: Vascular endothelial growth factor.

**Correspondence:**

Dr. Xiang Fan,
#10 Boyanghu Road, Jinghai District, Tianjin 301617, China.
E-mail: fanxiang_78@hotmail.com

**DOI:** 10.4103/pm.pm_541_19

**Access this article online**
**Website:** www.phcog.com
**Quick Response Code:**

## INTRODUCTION

Stroke is the major cerebrovascular disease which causes death and long-term disability in the world. The pathological mechanism of neuronal damage after ischemic stroke are mainly related to the release of excitatory amino acids; calcium overload; excessive production of free radicals; and peri-infarct depolarization, inflammation, and programmed cell death.[1] Although the cost of research and development of drugs for ischemic stroke has increased year-after-year, so far, only a few drugs have been clinically approved. Recombinant tissue plasminogen activator, which can dissolve the blood clot within 4.5 h after the onset of stroke, is still the only emergency treatment for ischemic stroke,[2] and there is no specific drug recommended during the recovery period of ischemic stroke. However, many studies have suggested that the therapeutic



**Cite this article as:** Yan C, Ma Y, Li L, Zhang M, Zhan J, Fan X. Effect and mechanism of ginsenoside Rg1 on synaptic plasticity of oxygen-glucose deprivation/reoxygenation-induced neuronal injury. Phcog Mag 2020;16:630-6.

targets of ischemic stroke should not only focus on the factors of resistance to injury but should also focus on endogenous neuroprotective mechanisms.[3] Previous studies demonstrate that synaptic plasticity is closely relevant to the Central Nervous System (CNS) development and neural repair after cerebral ischemic injury.[4]

GRg1 is the primary bioactive component of ginseng, which is a famous traditional Chinese medicine obtained from the root of *Panax ginseng* (CA Mey). It is used in the treatment of ischemic cardiovascular and cerebrovascular diseases. It can cross the blood brain barrier to enter the brain parenchyma after the oral administration of ginseng.[5] Previous studies have demonstrated that ginsenoside Rg1 shows protective effects on cerebral injury induced by ischemia/reperfusion. It significantly improved neurological function, attenuated the infarct volumes and brain edema, decreased the expression of proinflammatory cytokines, and decreased the contents of excitatory amino acids in focal ischemic stroke rats. It is known to mediate by suppressing the nuclear translocation of nuclear factor kappa B (NF-κB), as well as the phosphorylation of the inhibitory protein (IκBα), thereby increasing the expression of brain-derived neurotrophic factor (BDNF) in CA1 region of the hippocampus and activating the peroxisome proliferator-activated receptor gamma signaling.[6-8] Furthermore, ginsenoside Rg1 protects primary hippocampal neurons from ischemia/reperfusion-induced injury, which may be mediated by inhibiting calcium overload and by decreasing the activity of neuronal nitric oxide (NO) synthase.[9] Ginsenoside Rg1 rescued cortical neurons from apoptosis by stimulating Aβ25-35 and enhancing the neurite outgrowth through the downregulation of the NF-κB/NO pathway and activation of expression of AKT serine/threonine kinase and extracellular regulated protein kinases 1/2 (ERK1/2).[10,11]

Ginsenoside Rg1 demonstrated considerable protective effects on neurons injured by ischemia/reperfusion both in *in vitro* and *in vivo* conditions. However, the effect and mechanisms of action of ginsenoside Rg1 on the neural synaptic plasticity injured by ischemia/reperfusion have not yet been clarified. Therefore, in this study, we will establish the oxygen-glucose deprivation/reoxygenation (OGD/R)-injured primary cortical neurons model to mimic ischemia/reperfusion injury and investigate the effect and potential mechanisms of action of ginsenoside Rg1 on neural synaptic plasticity.

# MATERIALS AND METHODS

## Materials

Ginsenoside Rg1 was obtained from the National Institutes for Food and Drug Control (Beijing, China). Dulbecco's modified Eagle's medium (DMEM)/F12, B27, and trypsin were purchased from Invitrogen (Eugene, USA). CytoTox-ONETM Homogeneous Membrane Integrity Assay were obtained from Promega (Madison, WI, USA). Cell counting kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit was obtained from BD Biosciences (San Jose, CA, USA). Rat microtubule-associated protein (MAP)-2, BDNF, and insulin-like growth factors (IGF)-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from USCN (Wuhan, China). Rat growth-associated protein (GAP)-43, Tau, and vascular endothelial growth factor (VEGF) ELISA kits were purchased from Cusabio (Wuhan, China). TaqMan reverse transcription reagents were purchased from Applied Biosystems (Foster City, USA). Anti-Tau antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin (Ig) G was purchased from Invitrogen (Eugene, USA). HP RNA Isolation Kit, First Strand cDNA Synthesis Kit for real-time polymerase chain reaction (RT-PCR), and Transcriptor One-Step RT-PCR Kit were obtained from Roche (Basel, Switzerland).

## Preparation and culture of primary cortical neurons

Cerebral cortical neurons were isolated on day 16 from Wistar rat embryos in accordance with the previous literature.[12] In brief, cerebral cortices of embryos were dissected. After digestion, the cortex tissue homogenate was filtered through a 74 μm nylon sieve and centrifuged. The pellet obtained was resuspended in DMEM/F12 medium supplemented with 10% fetal bovine serum and plated onto poly-D-lysine-coated culture plates. After 4 h, the culture medium was changed to DMEM/F12 supplemented with 2% B-27. On days 7–8 after primary culture, neurons were used for further experiments.

## Establishment of oxygen-glucose deprivation/reoxygenation-injured neuron model

OGD/R model was established to mimic ischemia/reperfusion injury in accordance with the previous method.[12]. In brief, the culture medium was changed with glucose-free DMEM with ginsenoside Rg1 (0.1, 1, and 10 μM) and the neurons were incubated in the specialized hypoxia incubator chamber (Stem Cell, Canada) containing an anaerobic gas mixture (95% $N_2$ and 5% $CO_2$) at 37°C for 4 h. After OGD, the medium was changed with DMEM containing 4.5 mM glucose (pH 7.4) and the neurons were incubated in a regular $CO_2$ incubator for 2 h reoxygenation and glucose restoration. The control group were cultured with DMEM containing 4.5 mM glucose (pH 7.4) in a regular $CO_2$ incubator for 6 h.

## Measurement of cell viability and lactate dehydrogenase release

After OGD/R, culture medium and neurons were separated. CytoTox-ONETM homogeneous membrane integrity assay and CCK-8 assay were performed to measure the release of lactate dehydrogenase (LDH) in the culture medium and cell viability of neurons in accordance with assay's instructions, respectively.

## Measurement of apoptosis by flow cytometer

After OGD/R, neurons were harvested and incubated with Binding Buffer containing Annexin V-FITC and propidium iodide for 15 min. Cellular fluorescence was detected by flow cytometer with an excitation wavelength of 458 nm and emission wavelength of 560 nm in accordance with FITC Annexin V Apoptosis Detection Kit's instructions.

## Immunofluorescence analysis of axonal outgrowth

Immunofluorescence was performed following a standard method as previously described.[13] After OGD/R, neurons were fixed by 4% paraformaldehyde and immunostained with anti-Tau monoclonal antibody (1:200) as an axonal marker. Then, the cells were incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG (1:200). Four fields, which were positive for Tau immunofluorescence staining, were acquired using a 20 objective through the fluorescent microscope. The length of the axon for each neuron was measured using Image J software (National Institutes of Health (NIH), United States of America).

## Quantitative real-time polymerase chain reaction analysis

After OGD/R, total mRNA from neurons were extracted and reverse transcribed into cDNA in accordance with the previous method.[14] All primers were obtained from Sangon Biotech (Shanghai, China). Table 1 shows the forward and reverses primers for Bax, Bcl-2, BDNF, GAP-43,

**Table 1:** The forward and reverse primers

| Genes | Primer/probe | Primer/probe sequences (5′ to 3′) |
|---|---|---|
| Bax | Forward primer | 5′-TTGCTACAGGGTTTCATCCA-3′ |
| | Reverse primer | 5′-TGTTGTTGTCCAGTTCATCG-3′ |
| Bcl-2 | Forward primer | 5′-GAGCGTCAACAGGGAGATGT-3′ |
| | Reverse primer | 5′-CAGCCAGGAGAAATCAAACAG-3′ |
| BDNF | Forward primer | 5′-GCGGCAGATAAAAAGACT-3′ |
| | Reverse primer | 5′-CTTATGAATCGCCAGCCAAT-3′ |
| GAP-43 | Forward primer | 5′-CGACAGGATGAGGGTAAAGAAGA-3′ |
| | Reverse primer | 5′-GTGAGCAGGACAGGAGAGGAA -3′ |
| IGF-1 | Forward primer | 5′-TTGCGGGGCTGAGCTGGTGG-3′ |
| | Reverse primer | 5′-GCGGTGACGTGGCATTTTCT-3′ |
| MAP-2 | Forward primer | 5′-AAAGCGGCACCTCAACAC-3′ |
| | Reverse primer | 5′-CTCACTGGGCACCAAGAT-3′ |
| Tau | Forward primer | 5′ -CGGCGTAAGCAAAGACA -3′ |
| | Reverse primer | 5′ -TGTAGCCGCTTCGTTCT- 3′ |
| VEGF | Forward primer | 5′- CTTTCTGCTCTCTTGGGTGC-3′ |
| | Reverse primer | 5′-GTAGACGTCCATGAACTTCAC-3′ |
| β-actin | Forward primer | 5′-AGAGGGAAATCGTGC-3′ |
| | Reverse primer | 5′-CGATAGTGATGACCT-3′ |

VEGF: Vascular endothelial growth factor; BDNF: Brain derived neurotrophic factor; GAP-43: Growth associated protein-43; IGF-1: Insulin like growth factors 1; MAP-2: Microtubule-associated protein 2

IGF-1, MAP-2, Tau, VEGF, and β-actin. ABI 7500 RT-PCR System was used to amplify target genes, and 2-ΔΔCT method was used to analyze gene expression.

### Measurement of growth associated protein-43, microtubule-associated protein-2, tau, insulin like growth factors, brain derived neurotrophic factor, and vascular endothelial growth factor activity

After OGD/R, the culture medium was collected and the following tests performed: GAP-43, MAP-2, Tau, IGF, BDNF, and VEGF activity were measured using rat GAP-43, rat MAP-2, rat Tau, rat IGF-1, rat BDNF, and rat VEGF ELISA kits, respectively, in accordance with kit's instructions.

### Determination of cytosolic $Ca^{2+}$ concentration

Cytosolic $Ca^{2+}$ concentration was detected using the Rhod 2-AM probe in accordance with the previous method.[12] After OGD/R, neurons were incubated with Rhod 2-AM working solution (hanks balanced salt solution containing 4 μM Rhod 2-AM and 0.05% pluronic-F127) for 40 min. The cells were washed with D-Hank's, and cellular fluorescence was detected by a fluorescence microplate reader with excitation wavelength at 557 nm and emission wavelength at 581 nm.

### Statistical analysis

Results were expressed as mean ± standard deviation. Comparisons among groups were determined by one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

## RESULTS

### Ginsenoside Rg1 protected neurons from oxygen-glucose deprivation/reoxygenation injury

OGD/R decreased cell viability to 59.46% ± 4.41% and increased release of LDH from cells compared with the control group, treatment with ginsenoside Rg1 (0.1, 1, and 10 μM) significantly promoted cell viability and inhibited the release of LDH in a dose-dependent manner when compared to OGD/R group [Figure 1a and b]. OGD/R injury significantly augmented apoptosis rate from 6.13% ± 0.4% to 15.50% ± 0.92% at 2 h after reoxygenation, treatment with ginsenoside Rg1 (1 and 10 μM) declined apoptosis rate from 15.50% ± 0.92% to 13.4% ± 0.56% and 7.73% ± 0.67% in a dose-dependent manner [Figure 1c]. OGD/R injury dramatically decreased the axon length of neurons compared with the control group, ginsenoside Rg1 (1 and 10 μM) significantly increased the axon length compared with ODG/R group [Figure 1d].

### Ginsenoside Rg1 improved mRNA expression and activity of growth associated protein-43, microtubule-associated protein-2, and tau in neurons after oxygen-glucose deprivation/reoxygenation injury

OGD/R significantly increased the mRNA expression of GAP-43 to 1.39 ± 0.21 fold and decreased the mRNA expression of MAP-2 and Tau to 0.71 ± 0.17 and 0.34 ± 0.19 fold compared to that of the control group. Ginsenoside Rg1 improved GAP-43 mRNA expression and attenuated the reduction of MAP-2 and Tau mRNA expression in neurons induced by OGD/R injury [Figure 2a-c]. OGD/R remarkably increased the secretion of GAP-43 and inhibited the secretion of MAP-2 and Tau proteins in the culture medium, while 1 and 10 μM ginsenoside Rg1 treatment significantly increased the release and activity of GAP-43, MAP-2 and Tau in neurons after OGD/R [Figure 2d-f].

### Ginsenoside Rg1 improved mRNA expression and activity of insulin-like growth factors-1, brain-derived neurotrophic factor, and vascular endothelial growth factor in neurons after oxygen-glucose deprivation/reoxygenation injury

OGD/R significantly increased IGF-1 and VEGF mRNA expression to respectively 1.54 ± 0.41 and 1.42 ± 0.19 fold, inhibited BDNF mRNA expression to 0.89 ± 0.05 fold compared to that of control group. In this study, 10 μM ginsenoside Rg1 improved the mRNA expression of IGF-1 and VEGF and attenuated the reduction of BDNF mRNA expression in neurons after OGD/R [Figure 3a-c]. OGD/R injury increased the secretion of IGF-1 and VEGF and decreased BDNF secretion in the culture medium, whereas 1 and 10 μM ginsenoside Rg1 increased the secretion and activity of IGF-1, BDNF, and VEGF in neurons after OGD/R [Figure 3d-f].

### Effects of ginsenoside Rg1 on apoptosis related genes expression and cytosolic $Ca^{2+}$ concentration in neurons after oxygen-glucose deprivation/reoxygenation injury

OGD/R injury significantly downregulated the mRNA expression of Bcl-2, upregulated the mRNA expression of Bax, and decreased the ratio of Bcl-2/Bax gene expression. Treatment with 0.1, 1, and 10 μM ginsenoside Rg1 remarkably upregulated the Bcl-2 gene expression and 1 and 10 μM Ginsenoside Rg1 downregulated the Bax gene expression. Furthermore, ginsenoside Rg1 significantly upregulated the ratio of Bcl-2/Bax gene expression in neurons after OGD/R [Figure 4a-c]. OGD/R significantly increased cytosolic $Ca^{2+}$ concentration to 124.52% ± 6.43% compared with the control group. Treatment with 10 μM ginsenoside Rg1 significantly reduced cytosolic $Ca^{2+}$ concentration in neurons after OGD/R [Figures 4d].

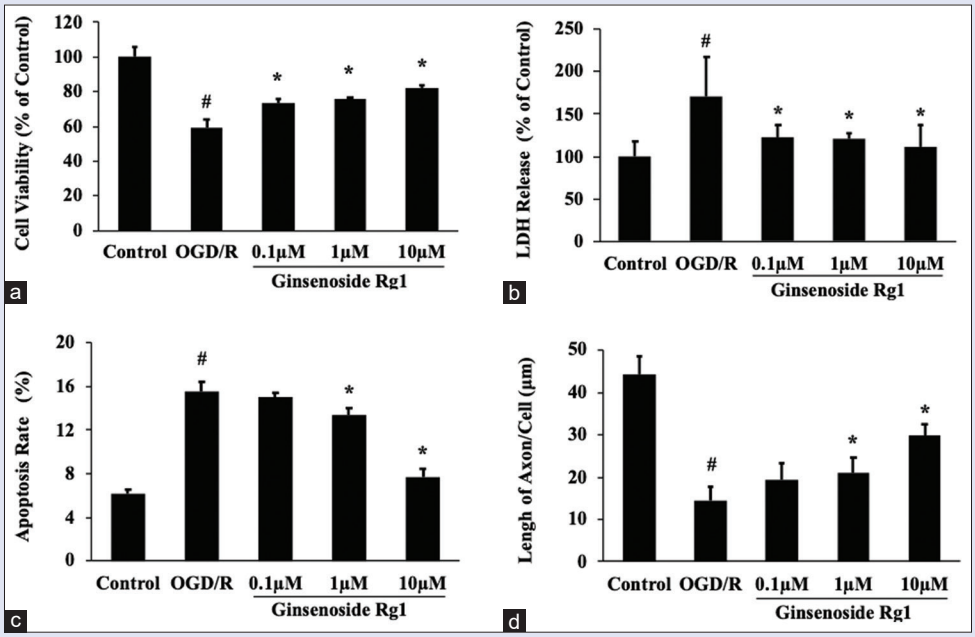



**Figure 1:** Protective effects of ginsenoside Rg1 on neurons after oxygen–glucose deprivation/reoxygenation injury. (a) Cell viability was determined using cell counting kit-8 assay. (b) Release of lactate dehydrogenase from neurons was measured using CytoTox-ONE™ homogeneous membrane integrity assay. (c) Apoptosis of neurons were stained by Annexin V-FITC/Propidium Iodide and detected by flow cytometry. (d) The length of axon was detected by tau immunofluorescence staining. Data are shown as mean ± standard deviation $n = 6$. $^{\#}P < 0.05$ compared with control; $^{*}P < 0.05$ compared with oxygen–glucose deprivation/reoxygenation

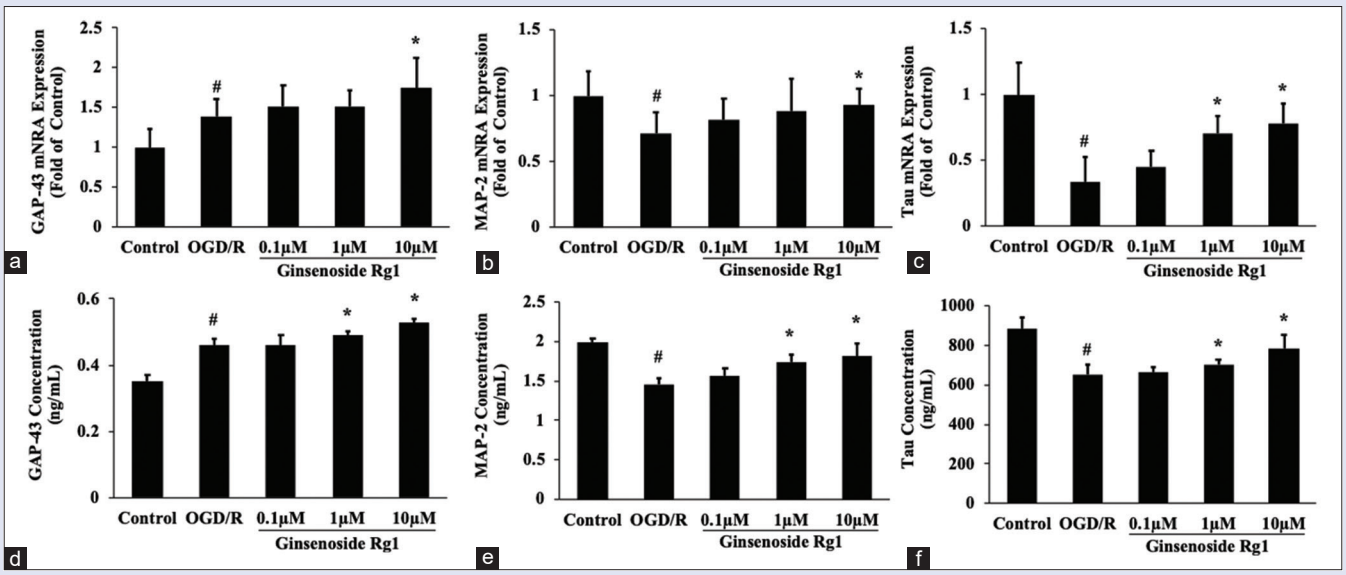



**Figure 2:** Effects of ginsenoside Rg1 on growth associated protein-43, microtubule-associated protein-2 and Tau expression in neurons after oxygen–glucose deprivation/reoxygenation injury. (a) The expression of growth associated protein -43 gene was measured by real-time polymerase chain reaction. (b) The expression of microtubule-associated protein-2 gene was measured by real-time polymerase chain reaction. (c) The expression of Tau gene was measured by real-time polymerase chain reaction. (d) Growth associated protein-43 activity detected by enzyme-linked immunosorbent assay. (e) Microtubule-associated protein-2 activity detected by enzyme-linked immunosorbent assay. (f) Tau activity detected by enzyme-linked immunosorbent assay. Data are shown as mean ± standard deviation $n = 6$. $^{\#}P < 0.05$ compared with control; $^{*}P < 0.05$ compared with oxygen–glucose deprivation/reoxygenation

## DISCUSSION

Ischemic stroke injury is accompanied with the secretion of excitatory amino acids, calcium overload, excessive free radical production and depolarization, and inflammation and programmed cell death around the infarct. The cascade of reactions leading to ischemia is responsible for for the destruction of synaptic structure and loss of synaptic transmission, which will cause neurological dysfunction. However, the damaged neurons cannot regenerate in the CNS, and the residual neurons can send out dendrites or axons to play a compensatory role, although this compensatory effect is very weak. In this study, ginsenoside Rg1 was used to treat the injured neurons after ischemia/reperfusion. The results

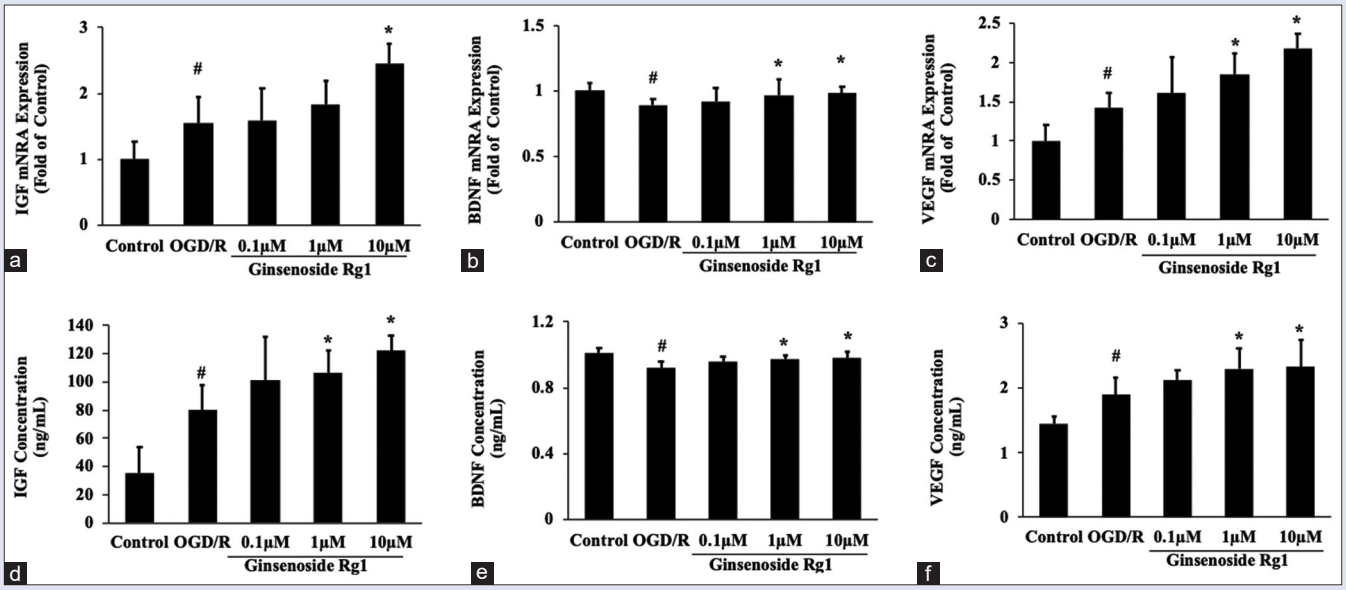



**Figure 3:** Effects of ginsenoside Rg1 on insulin like growth factors-1, brain derived neurotrophic factor and vascular endothelial growth factor expression in neurons after oxygen–glucose deprivation/reoxygenation injury. (a) The expression of insulin like growth factors-1 gene was measured by real-time polymerase chain reaction. (b) The expression of brain derived neurotrophic factor gene was measured by real-time polymerase chain reaction. (c) The expression of vascular endothelial growth factor gene was measured by real-time polymerase chain reaction. (d) Insulin like growth factors-1 activity detected by enzyme-linked immunosorbent assay. (e) Brain derived neurotrophic factor activity detected by enzyme-linked immunosorbent assay. (f) Vascular endothelial growth factor activity detected by enzyme-linked immunosorbent assay. Data are shown as mean ± standard deviation $n = 6$. $^{\#}P < 0.05$ compared with control; $^{*}P < 0.05$ compared with oxygen–glucose deprivation/reoxygenation

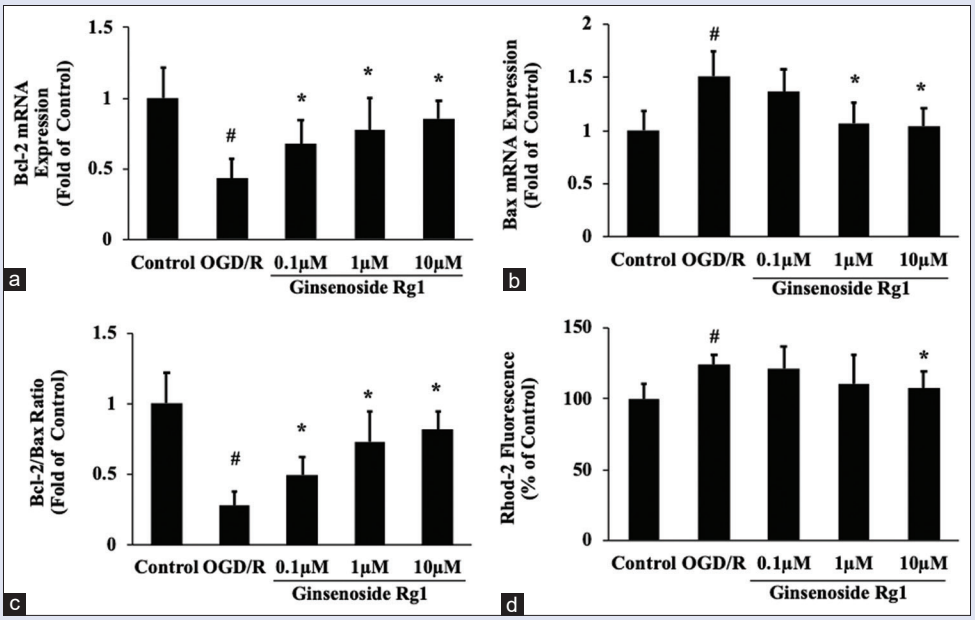



**Figure 4:** Effects of ginsenoside Rg1 on apoptosis related genes expression and cytosolic $Ca^{2+}$ concentration in neurons after oxygen–glucose deprivation/reoxygenation injury. (a) The expression of Bcl-2 gene was measured by real-time-polymerase chain reaction. (b) The expression of Bax gene was measured by real-time-polymerase chain reaction. (c) The ratio of Bcl-2/Bax gene was measured by real-time-polymerase chain reaction. (d) Cytosolic $Ca^{2+}$ was measured by Rhod-2 fluorescence staining. Data are shown as mean ± standard deviation $n = 6$. $^{\#}P < 0.05$ compared with control; $^{*}P < 0.05$ compared with oxygen–glucose deprivation/reoxygenation

demonstrate that ginsenoside Rg1 can improve cell viability, decrease rate of apoptosis, and promote axonal regeneration and neuronal remodeling to protect neurons from OGD/R injury [Figure 1]. It can be seen that enhanced synaptic plasticity is extremely important in the treatment of ischemic stroke. Synaptic plasticity is an important basis for maintaining brain function. Structural proteins synthesized during neurite regeneration, such as GAP-43, MAP-2, and Tau, have significant regulatory effects on axonal growth, formation of new synapse and functional improvement, which are also intrinsic factors that affect the regeneration of neurons.

GAP-43, as a specific membrane phosphoprotein in the axon growth cone of the neuron, plays an important role in promoting the nerve cell growth, axonal regeneration, and synaptic remodeling.[15] GAP-43 is a molecular marker of axonal growth and plasticity, which is expressed at high levels during neuronal development and axon growth. Tau is a neural microtubule-associated cytoskeletal protein, which is wildly distributed in neurons under normal condition. Tau is mainly expressed in the axons of neurons in the brain.[16] MAP-2 is primarily found in the dendrites and cell bodies of neurons, which is a neurocytoskeleton component and participates in dendritic differentiation, neurite growth, and polarity formation of neurons.[17] Therefore, GAP-43, MAP-2, and Tau are considered to be related proteins of nerve growth and repair and molecular markers of nerve remodeling. In this study, ginsenoside Rg1 remarkably upregulated the mRNA expression of GAP-43, MAP-2, and Tau and secretion of GAP-43, MAP-2, and Tau protein in the culture medium compared with OGD/R group [Figure 2]. It can be seen that appropriate concentration of ginsenoside Rg1 can promote axonal regeneration and enhance synaptic plasticity and play a neuroprotective role through the regulation of expression of synaptic remodeling proteins, such as GAP-43, MAP-2, and Tau.

Neurotrophic factors also play a critical role in regulating synaptic plasticity. BDNF, as one of the neurotrophic factors, can not only transmit the signal between neurons and stimulating factors but also affect the interaction between neurons in the neural network and participate in the regulation of synaptic formation; however, the mechanism is diverse.[18] BDNF induces the formation of the premature synapse by promoting the binding of GIT1 and CaMKIIβ. Serine at position 419 of GIT1 is not only the key to the interaction between GIT1 and CaMKIIβ, but also an important site for BDNF to promote phosphorylation of CaMKIIβ and formation of premature synapse within cortical neurons.[19] BDNF stimulated the neurons for a short time to increase the number of synapses through Trkb/PI3K (tyrosine-related kinase B/phosphatidylinositide 3-kinase) pathway, whereas BDNF stimulated the neurons for a long time (12–24 h) regulates the synaptic formation via the Trkb/ERK-1/2 pathway.[20,21] The previous study also reported that BDNF promoted axonal regeneration by increasing the levels of cAMP, activating protein kinase A, and inhibiting RhoA signaling pathways.[22] In this study, ginsenoside Rg1 significantly increase the mRNA and protein expression of BDNF in neurons after OGD/R injury. This suggests that ginsenoside Rg1 promotes axonal regeneration and neuronal remodeling by upregulating the expression of BDNF.

IGF-1 is a neurotrophic hormone. It promotes the growth of neuronal cells and prevents cell death. It plays a crucial role in the development and maturation of CNS. IGF-1 can regulate cell growth and differentiation, reduce the apoptotic process of injured cells, and restore damaged cells.[23] It has also been reported that the high expression of IGF-1 is positively correlated with the process of axonal protrusion buds and formation of synaptic fibers of neurons.[24] In this study, the expression of IGF-1 mRNA and protein was significantly upregulated after OGD/R when compared to control group, which is consistent previous reports.[25] In this study, ginsenoside Rg1 could significantly increase the expression of endogenous IGF-1 in neuron after OGD/R injury, which is positively correlated with the regulation of neuronal axon and synaptic plasticity.

Recent studies on the neuroprotective effect of VEGF have attracted increasing attention as a highly specific vascular endothelial cell mitotic factor, which not only promotes angiogenesis and facilitates the establishment of collateral circulation but also exerts neurotrophic and regenerative effects.[26-28] In this study, ginsenoside Rg1 might significantly upregulate VEGF expression in neurons injured by OGD/R, which suggests that ginsenoside Rg1 plays a pivot role in axonal regeneration and neuronal remodeling by increasing VEGF expression.

$Ca^{2+}$ signal transduction in neurons plays a key role in the development and plasticity of CNS.[29] $Ca^{2+}$ can affect the growth of nerve pyramids, neuronal migration, axon and dendrite extension, and synaptic plasticity.[30] However, calcium overload in the CNS could cause abnormalities of various functions such as neuroplasticity and protein synthesis. In this study, the intracellular $Ca^{2+}$ concentration of neurons in OGD/R group was remarkably increased when compared to the control group, which may be one of the important factors leading to abnormal synaptic plasticity after cerebral ischemia/reperfusion injury. However, intracellular calcium overload was significantly inhibited by ginsenoside Rg1 administration compared to OGD/R group. Meanwhile, the expression of Bcl-2 was upregulated and the expression of Bax was downregulated and neuronal apoptosis was alleviated by ginsenoside Rg1 administration, which suggest that ginsenoside Rg1 regulates synaptic plasticity by inhibiting intracellular $Ca^{2+}$ overload and neuronal apoptosis.

The above preliminarily results indicate that ginsenoside Rg1 can promote axonal regeneration and neuronal remodeling after OGD/R injury. The mechanism may be mediated by the upregulation of endogenous neurotrophic factors, such as IGF-1, BDNF, and VEGF, inhibition of intracellular $Ca^{2+}$ overload, and regulation of Bcl-2 and Bax expression, thereby triggering the overexpression of structural proteins synthesized during neurite regeneration such as GAP-43, MAP-2, and Tau.

However, this study has some limitations and caveats that should also be pointed out. First, the signaling pathway that is involved in the synaptic plasticity is extremely complex; further experiments are needed to confirm by which signaling pathway that ginsenoside Rg1 promotes neuronal remodeling after OGD/R injury. Second, in this study, we found that ginsenoside Rg1 promotes axonal regeneration and neuronal remodeling after OGD/R injury under *in vitro* conditions. Future studies should explore if ginsenoside Rg1 promotes neuronal remodeling after ischemic stroke under *in vivo* conditions and its potential mechanism of action needs to be determined.

## CONCLUSION

Ginsenoside Rg1 can promote axonal regeneration and neuronal remodeling after OGD/R injury through the upregulation of endogenous neurotrophic factors and synaptic remodeling proteins, inhibiting intracellular $Ca^{2+}$ overload, and regulating the expression of apoptotic genes, thereby protecting neurons from OGD/R injury.


### Financial support and sponsorship

This work was supported by the National Natural Science Foundation of China (No: 81622051), Natural Science Foundation of Tianjin City (No: 15JCYBJC54800), and Training Program Foundation for Innovative Research Team of Higher Education in Tianjin during the 13th Five-Year Plan Period (No: TD13-5050).


### Conflicts of interest

There are no conflicts of interest.

## REFERENCES


1. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 2003;4:399-415.
2. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, *et al.* Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317-29.
3. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: Mechanisms in search of treatments. Neuron 2010;67:181-98.
4. Balbinot G, Schuch CP. Compensatory relearning following stroke: Cellular and plasticity

mechanisms in rodents. Front Neurosci 2018;12:1023.

5. Zhao YN, Shao X, Ouyang LF, Chen L, Gu L. Qualitative detection of ginsenosides in brain tissues after oral administration of high-purity ginseng total saponins by using polyclonal antibody against ginsenosides. Chin J Nat Med 2018;16:175-83.
6. Zheng T, Jiang H, Jin R, Zhao Y, Bai Y, Xu H, *et al.* Ginsenoside Rg1 attenuates protein aggregation and inflammatory response following cerebral ischemia and reperfusion injury. Eur J Pharmacol 2019;853:65-73.
7. Li Y, Guan Y, Wang Y, Yu CL, Zhai FG, Guan L ×. Neuroprotective effect of the ginsenoside rg1 on cerebral ischemic injury *in vivo* and *in vitro* is mediated by PPARγ-regulated antioxidative and anti-inflammatory pathways. Evid Based Complement Alternat Med 2017;2017:7842082.
8. Wang L, Zhao H, Zhai ZZ, Qu LX. Protective effect and mechanism of ginsenoside Rg1 in cerebral ischaemia-reperfusion injury in mice. Biomed Pharmacother 2018;99:876-82.
9. He Q, Sun J, Wang Q, Wang W, He B. Neuroprotective effects of ginsenoside Rg1 against oxygen-glucose deprivation in cultured hippocampal neurons. J Chin Med Assoc 2014;77:142-9.
10. Wu J, Yang H, Zhao Q, Zhang X, Lou Y. Ginsenoside Rg1 exerts a protective effect against Abeta (2)(5)(-)(3)(5)-induced toxicity in primary cultured rat cortical neurons through the NF-kappaB/NO pathway. Int J Mol Med 2016;37:781-8.
11. Huang L, Liu LF, Liu J, Dou L, Wang GY, Liu XQ, *et al.* Ginsenoside Rg1 protects against neurodegeneration by inducing neurite outgrowth in cultured hippocampal neurons. Neural Regen Res 2016;11:319-25.
12. Ma Y, Zhao P, Zhu J, Yan C, Li L, Zhang H, *et al.* Naoxintong protects primary neurons from oxygen-glucose deprivation/reoxygenation induced injury through PI3K-Akt signaling pathway. Evid Based Complement Alternat Med 2016;2016:5815946.
13. Fan X, Lo EH, Wang X. Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in type 1 diabetic rats. Stroke 2013;44:745-52.
14. Zhang M, Ma Y, Chai L, Mao H, Zhang J, Fan X. Storax protected oxygen-glucose deprivation/reoxygenation induced primary astrocyte injury by inhibiting NF-κB activation *in vitro*. Front Pharmacol 2018;9:1527.
15. Oestreicher AB, de Graan PN, Gispen WH, Verhaagen J, Schrama LH. B-50, the growth associated protein-43: Modulation of cell morphology and communication in the nervous system. Prog Neurobiol 1997;53:627-86.
16. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. Acta Neuropathol 2017;133:665-704.
17. Johnson GV, Jope RS. The role of microtubule-associated protein 2 (MAP-2) in neuronal growth, plasticity, and degeneration. J Neurosci Res 1992;33:505-12.
18. de Vincenti AP, Ríos AS, Paratcha G, Ledda F. Mechanisms that modulate and diversify BDNF functions: Implications for hippocampal synaptic plasticity. Front Cell Neurosci 2019;13:135.
19. Zhang Z, Zhou W, Fan J, Ren Y, Yin G. G-protein-coupled receptor kinase interactor-1 serine 419 accelerates premature synapse formation in cortical neurons by interacting with Ca (2+)/calmodulin-dependent protein kinase IIβ. Brain Res Bull 2013;95:70-7.
20. Luikart BW, Zhang W, Wayman GA, Kwon CH, Westbrook GL, Parada LF. Neurotrophin-dependent dendritic filopodial motility: A convergence on PI3K signaling. J Neurosci 2008;28:7006-12.
21. Alonso M, Medina JH, Pozzo-Miller L. ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons. Learn Mem 2004;11:172-8.
22. Park H, Poo MM. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 2013;14:7-23.
23. Huat TJ, Khan AA, Pati S, Mustafa Z, Abdullah JM, Jaafar H. IGF-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells. BMC Neurosci 2014;15:91.
24. Dyer AH, Vahdatpour C, Sanfeliu A, Tropea D. The role of insulin-like growth factor 1 (IGF-1) in brain development, maturation and neuroplasticity. Neuroscience 2016;325:89-99.
25. Hwang IK, Yoo KY, Park SK, An SJ, Lee JY, Choi SY, *et al.* Expression and changes of endogenous insulin-like growth factor-1 in neurons and glia in the gerbil hippocampus and dentate gyrus after ischemic insult. Neurochem Int 2004;45:149-56.
26. Ma Y, Zechariah A, Qu Y, Hermann DM. Effects of vascular endothelial growth factor in ischemic stroke. J Neurosci Res 2012;90:1873-82.
27. Dzietko M, Derugin N, Wendland MF, Vexler ZS, Ferriero DM. Delayed VEGF treatment enhances angiogenesis and recovery after neonatal focal rodent stroke. Transl Stroke Res 2013;4:189-200.
28. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, *et al.* VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest 2003;111:1843-51.
29. Bito H, Takemoto-Kimura S. Ca (2+)/CREB/CBP-dependent gene regulation: A shared mechanism critical in long-term synaptic plasticity and neuronal survival. Cell Calcium 2003;34:425-30.
30. Cavazzini M, Bliss T, Emptage N. Ca2+and synaptic plasticity. Cell Calcium 2005;38:355-67.