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

ORIGINAL ARTICLE

Neuroprotective Compounds from the Embryo of *Nelumbo nucifera* Seeds

Jin Bae Weon, Choong Je Ma[1]

Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, [1]Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, South Korea

Submitted: 28-Nov-2018 Revised: 02-Jan-2019 Accepted: 31-Oct-2019 Published: 15-Jun-2020

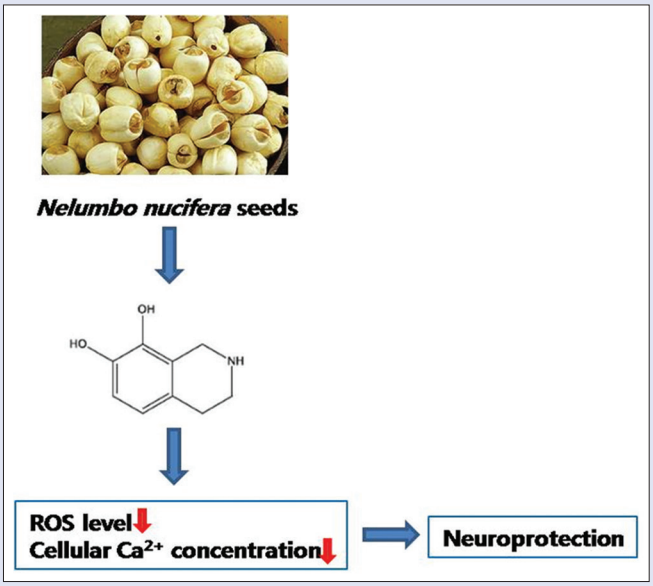
ABSTRACT

Background: Previous studies have shown the cognitive effect of the embryo of *Nelumbo nucifera* on scopolamine-induced memory impairment and neuroprotective effect against glutamate-injured neurotoxicity in HT-22 cells. **Objectives:** The present study was designed for the purpose of evaluating the neuroprotective activity of compounds isolated from the embryo of *N. nucifera* seeds on glutamate-induced cell death in HT-22 cells. **Materials and Methods:** We isolated compound from the embryo of *N. nucifera* using various chromatograms and confirmed chemical structure by various spectroscopy. The neuroprotective effects of the compounds against glutamate-induced cell death in HT-22 cells were investigated using an (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **Results:** We isolated 6 compounds, 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinoline diol (2), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl -7-Isoquinolinol(3), 1-(3,4,5-trihydroxyphenyl)-ethanone(4), 1-(2,3,5,6-tetrahydroxyphenyl)-ethanone(5), 3-(prop-1-enyl) benzene-1,2,4,5-tetrol (6) from the embryo of *N. nucifera*. Among these compounds, 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquino linediol significantly decreased glutamate-induced cell death in HT-22 cells. In addition, these compounds exacerbated the reactive oxygen species level and intracellular Ca^{2+} accumulation. **Conclusion:** The neuroprotective efficacy of 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquinolinediolmay be related to their antioxidative effect.

Key words: 1,2,3,4-tetrahydro-7,8-isoquinolinediol, Ca^{2+} accumulation, embryo of *Nelumbo nucifera seeds*, neuroprotective effect, reactive oxygen species level

SUMMARY

- Two compounds isolated from *Nelumbo nucifera*, 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxy phenyl) methyl]-2-methyl-6,7-isoquinolinediol, showed potent neuroprotective activity in HT-22 cells by the inhibition of reactive oxygen species level and Ca^{2+} concentration.



Abbreviations used: AD: Alzheimer's disease; Aβ: Amyloid beta plaque deposit; NFT: Neurofibrillary tangles; ROS: Reactive oxygen species

Correspondence:

Prof. Choong Je Ma,
Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Kangwondaehak-gil 1, Chuncheon 24341, South Korea.
E-mail: cjma@kangwon.ac.kr
DOI: 10.4103/pm.pm_613_18

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

INTRODUCTION

Neurodegenerative disorders are characterized by the loss of neuronal structure and function including cell injury and memory dysfunction.[1] The most common disease result from neurodegenerative disorder is Alzheimer's disease (AD), and it is associated with pathogenesis, such as amyloid-beta plaque formation and neurofibrillary tangles deposits.[2] Oxidative stress such as lipid peroxidation, free radical synthesis, and protein and DNA oxidation contribute to the development of AD.[3]

Glutamate is an excitatory neurotransmitter and contributes to neurodegenerative diseases such as AD and Parkinson's disease.[4] Excessive glutamate can be excitotoxic and result in oxidative stress by increasing the amount of reactive oxygen species (ROS) and intracellular calcium (Ca^{2+}) concentration.[5]

Nelumbo nucifera Gaertneris an aquatic plant from the Nelumbonaceae family and is conventionally used in Korean, Chinese, and Japanese medicine and food to alleviate fever and arrest bleeding.[6] Almost all parts of *N. nucifera*, such as leaves, flowers, and rhizomes are used as medicine. In a previous study, the embryo of *N. nucifera* seeds has

Cite this article as: Weon JB, Ma CJ. Neuroprotective compounds from the embryo of *Nelumbo nucifera* seeds. Phcog Mag 2020;16:329-35.

shown a sedative effect in the mouse.[7] The embryo of *N. nucifera* seeds consists of bisbenzylisoquinoline alkaloids, benzylisoquinoline alkaloids, aporphine, and proaporphine alkaloids.[8-11] Neferine (bisbenzylisoquinoline alkaloid) is known as the major active compound in the embryo. Previous studies have determined neferine to have anti-cancer, anti-amnesic, anti-depressant, and anti-fibrotic effects.[12-15] In addition, neferine has been reported to attenuate the amount of mutant huntingtin and its toxicity in PC-12 cells.[16]

In the previous study, we found that the embryo of *N. nucifera* seeds attenuated scopolamine-induced memory impairment in mice due to acetylcholinesterase inhibition and protected HT-22 neuronal cells from glutamate-induced oxidative stress through the reduction of ROS production and the inhibition of intercellular Ca^{2+} accumulation.[17]

Here, we isolated compounds from the embryo of *N. nucifera* seeds and evaluated investigated their neuroprotective activity to confirm the active compounds.

The immortalized mouse hippocampal cell line, HT22, provided an *in vitro* experimental model with which to elucidate the mechanisms related to glutamate-induced cell death.[18] The neuroprotective effect of fractions of the embryo of *N. nucifera* seeds and the isolated compounds were evaluated on glutamate-induced cell death in HT-22 cells using an MTT assay.

MATERIALS AND METHODS

Plant material

The embryos of *N. nucifera* seed were obtained from Wildlife Genetic Resources Center, National Institute of Biological Resources (Incheon, Korea). Plant material was authenticated by Dr. Hee Jeong Yang, a professor of the College of Pharmacy, Kangwon National University (Chuncheon, Korea) and voucher specimens (NO. CJ129M) were been deposited in Kangwon National University (Chuncheon, Korea).

Extraction and isolation

The embryo of *N. nucifera* seed (15.75 g) was extracted in 1.57 L of 80% methanol for 90 min in 2 L volumetric flask. This extraction was performed in ultrasonicator (Branson 5510, 40 KHz) with three times by ultrasonication-assisted extraction at room temperature. The extract was evaporated and suspended in water. The suspension was partitioned with n-hexane, $CHCl_3$, and ethyl acetate (EtOAc). Then, the respective n-hexane (0.76 g), $CHCl_3$ (1.00 g), and EtOAc (3.06 g) fractions were obtained. The EtOAc fraction (36.86 g) was loaded to Sephadex LH-20 chromatography under the condition of the mobile phase of MeOH: Water (4:1) to obtain five fractions (A–E). Compounds 1–3 were isolated from fraction C by preparative high-performance liquid chromatography (HPLC) on an YMC C_{18} (250 mm × 10 mmI.D. S-5 μm) column. The mobile phase consisted of water and acetonitrile with gradient system.

Compounds 4–6 were isolated from fraction D by preparative HPLC on a LUNA (250 mm × 10 mmI.D. S-5 μm) column. The mobile phase consisted of 0.1% Trifluoroacetic acid (TFA) and acetonitrile with gradient system.

Cell viability

HT-22 cells, mouse hippocampal cells, were cultured in Dulbecco's Modified Eagle Medium (DMEM) media filtered through 0.25 μm membrane filters, added with 10% (v/v) fetal bovine serum as a supplement, 1% penicillin/streptomycin as antibiotics, $NaHCO_3$ (2 mg/ml), and 15 mM HEPES. The cells were incubated at 37°C. CO_2 incubator containing 5% of CO_2 was used to incubate HT-22 cells.

Cell viability was evaluated by MTT assay as reported by the previous method with minor modifications. HT-22 cells were seed in 48 wells plate at a density of 6.7×10^4 cells/300 μL and cultured for 24 h at 37°C/5% CO_2. After incubation, 1, 10, and 100 μM of compounds and 50 μg/ml of Trolox (positive control) and glutamate were treated and incubation for 24 h. Then, 1 mg/ml of MTT solution was added in the HT-22 cells. After 3 h, dimethyl sulfoxide was treated to dissolve MTT-formazan crystals in each well and the optical density of viable cells was measured at 570 nm by ELISA reader. Cell viability was evaluated by the relative protection ratio (%). Relative protection (%) was obtained from the following formula: (OD of glutamate + drug-treated group − OD of the glutamate-treated group)/(OD of the control group − OD of the glutamate-treated group).

Reactive oxygen species generation

ROS production was evaluated using 2'7'-dichlorofluorescein diacetate dye (DCF-DA). HT-22 cells were added onto 48-well plate, and 1, 10, and 100 μM of compounds were treated with 2 mM glutamate for 8 h. Then, DMEM media were changed to DMEM without phenol red and 10 μM of DCF-DA was treated to the cells for 30 min. After incubation, cells were cleaned with PBS buffer. After that, cells were extracted with Triton X-100 (1%) in PBS buffer for 10 min at 37°C. Fluorescence was measured with the following excitation at 490 nm and emission at 525 nm.

Calcium (Ca^{2+}) measurement

Cytosolic Ca^{2+} concentration was evaluated using the fluorescent dye, Fura-2AM in HT-22 cells. Cells were loaded onto 48-well plate and cultured for 24 h. After incubation, 1, 10, and 100 μM of compounds, 2 μM Fura-AM and glutamate was treated to each well for 20 min. Then, cells were washed with HEPES buffer and stored for 1 h. Ca^{2+} level was evaluated by fluorescence (excitation wavelength at 380 nm and fixed emission at 510 nm).

Statistics

The results were described as means ± standard error of the mean and statistical analysis was accomplished with one-way analysis of variance with subsequent Tukey's *post hoc* test. As a result, statistical significance was evaluated at $P < 0.05$, 0.01, and 0.001.

RESULTS

Isolation and characterization of compounds from embryo of *Nelumbo nucifera* seed

Three isoquinolinediols and three phenolic compounds were isolated from the EtOAc fraction of the embryo of *N. nucifera* seeds. The structures of the compounds were elucidated using 1D-NMR experiments and were identified by comparison of the spectral data with those from the Pubchem database and previous studies.[19-22] The six compounds were identified as 1,2,3,4-tetrahydro-7,8-Isoquinolinediol (1),1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol(2),1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-7-Isoquinolinol(3),1-(3,4,5-trihydroxyphenyl)-ethanone (4), 1-(2,3,5,6-tetrahydroxyphenyl)-ethanone (5) and 3-(prop-1-enyl) benzene-1,2,4,5-tetrol (6) [Figure 1 and Tables 1-6].

Neuroprotective activity of compounds on glutamate-injured neurotoxicity in HT-22 cells

We investigated the neuroprotective effect of the partitioned fractions (n-hexane, $CHCl_3$, and EtOAc) on HT-22 cells treated with glutamate.

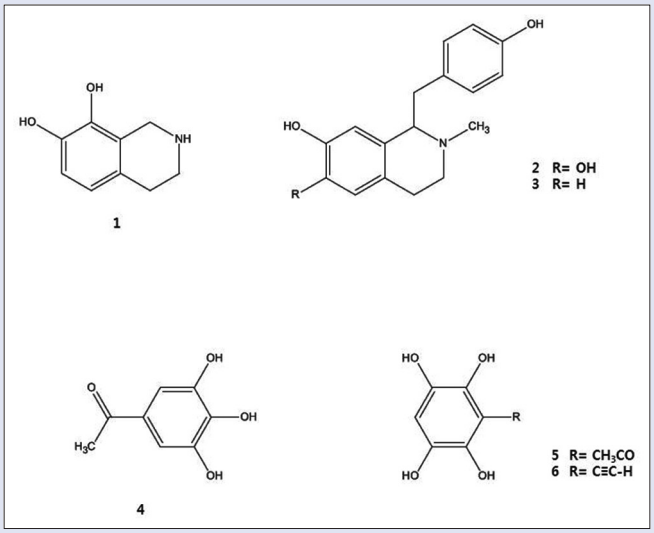


Figure 1: Chemical structure of compounds extracted from *Nelumbo nucifera* seeds. 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (2), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl] -2-methyl-7-Isoquinolinol (3), 1-(3,4,5-trihydroxyphenyl)-ethanone (4), 1-(2,3,5,6-tetrahydroxyphenyl)-ethanone (5), 3-(prop-1-enyl) benzene -1,2,4,5-tetrol (6)

Among the partitioned fractions, the EtOAc fraction showed significant protective effects against glutamate-induced cell death, with a relative protective ratio of 83.05 ± 12.64% at 10 μg/ml) [Figure 2]. We, therefore, isolated six compounds from the EtOAc fraction. Evaluation of the neuroprotective effect of six compounds was performed in HT-22 cells. Among these, 1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol exhibited a significant neuroprotective effect on the glutamate-induced neurotoxicity in HT-22 cells, with relative protection of 34.48 ± 7.58% and 18.18 ± 6.58% at 1 μM, respectively [Figure 3].

Effect of compounds on reactive oxygen species production

Here, we also investigate the effect of 1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1 -[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquinolinediolon the inhibition of ROS production using the fluorescent dye, DCF-DA. We found that ROS levels were increased by glutamate treatment in HT-22 cells. 1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquinolinediol effectively decreased glutamate-induced ROS production by 116.74 ± 4.35% (1 μM) and 121.50 ± 5.37% (1 μM) [Figure 4].

Effect of compounds on intracellular Ca^{2+} production

Intracellular Ca^{2+} production was investigated using Fura-AM to demonstrate whether 1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquinolinediol inhibited intracellular Ca^{2+} concentration in HT-22 cells. 1,2,3,4-tetrahydro -7,8-Isoquinolinediol (116.24 ± 2.42% at 1 μM) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (112.29 ± 5.39% at 1 μM) significantly decreased intracellular Ca^{2+} concentration in HT-22 cells [Figure 5].

Table 1: 1,2,3,4-tetrahydro-7,8-isoquinolinediol NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	9.82
OH aromatic C-OH	9.42
NH	2.0
CH_2	3.81
CH 1-benzene	2.36
CH 1-benzene	6.56
CH_2	2.97
C-13 NMR data	
C1	144.1
C2	142.1
C3	43.3
C4	44.3
C5	124.2
C6	130.1
C7	114.2
C8	121.5
C9	28.4

Table 2: 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	9.48
OH aromatic C-OH	9.48
OH aromatic C-OH	9.43
CH	4.29
CH_2	2.74, 2.64
CH 1-benzene	6.70
CH 1-benzene	6.81
CH 1-benzene	7.26
CH 1-benzene	7.12
CH 1-benzene	6.81
CH 1-benzene	7.12
CH 1-benzene	6.70
CH 1-benzene	7.12
CH_2	2.77, 2.73
CH_3	0.86
CH_2	1.37
C-13 NMR data	
C1	142.6
C2	144.1
C3	155.7
C4	64.6
C5	47.5
C6	129.8
C7	128.1
C8	132.0
C9	115.8
C10	113.5
C11	114.5
C12	130.2
C13	115.8
C14	130.2
C15	27.3
C16	42.5
C17	39.7

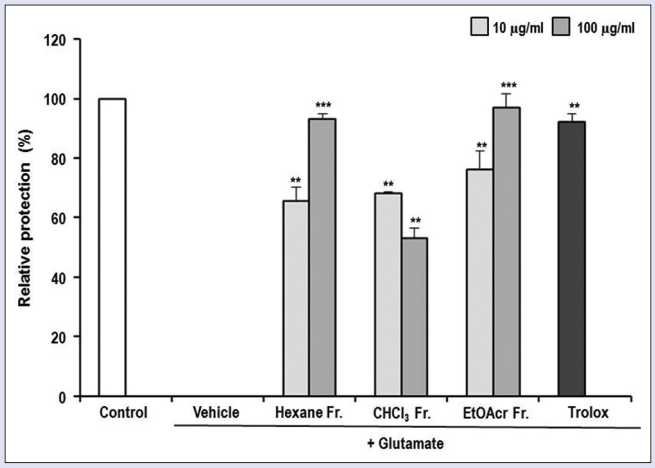


Figure 2: Neuroprotective effect of the fractions (hexane, chloroform, ethyl acetate, butanol) of *Nelumbo nucifera* seeds. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P < 0.01$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the glutamate-treated group

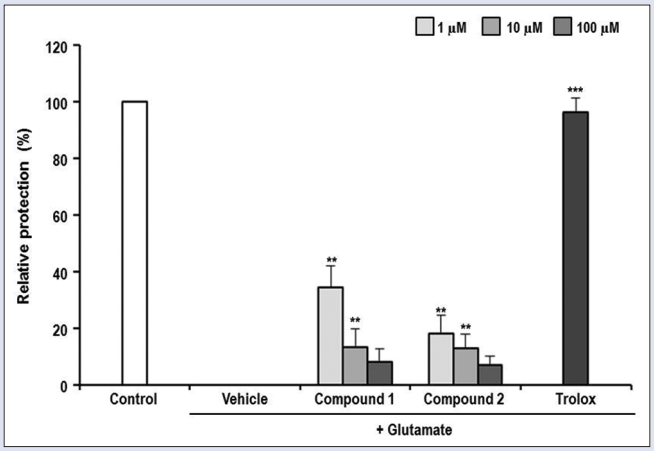


Figure 3: Neuroprotective effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl -6,7-Isoquinolinediol (2) on glutamate-induced cell death in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P < 0.01$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus the glutamate-treated group

DISCUSSION

We confirmed the cognitive enhancing effect of the embryo of *N. nucifera* seeds in the mouse.[7] Neuroprotective effect is correlated with cognitive enhancing effect. We isolated compounds in the embryo of *N. nucifera* seeds to identify neuroprotective compounds. EtOAc fraction was separated to obtain six compounds, 1,2,3,4-tetrahydro-7,8-Isoquinolinediol (1), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7- Isoquinolinediol (2), 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-7-Isoquinolinol (3),1-(3,4,5-trihydroxyphenyl)-ethanone (4), 1-(2,3,5,6-tetrahydroxyphenyl) -ethanone(5), 3-(prop -1-enyl) benzene-1,2,4,5-tetrol (6) from embryo of *N. nucifera* seed.

Six compounds were the first to be isolated from the embryo of *N. nucifera* seeds; however, these have not been reported to have neuroprotective effects. The present study demonstrates that compounds isolated from the extract of the embryo of *N. nucifera* seeds excert potent neuroprotection on glutamate-injured mouse hippocampal HT-22 cells by oxidative stress.

Glutamate inhibits cystine uptake through the cystine/glutamate antiporter and reduces antioxidants and glutathione levels.[23] ROS generation and Ca^{2+} influx into neuronal cells via N-methyl-D-aspartate (NMDA) receptors are also involved.[24,25] Increased activation of NMDA receptors elevates the intracellular Ca^{2+} concentration and results in the depolarization of the mitochondrial membrane by ROS production.[26] Accumulation of ROS can result in DNA impairment, protein oxidation, and lipid peroxidation in neuronal cells.[27] Oxidative stress is known to cause DNA damage, which eventually activates poly (ADP-ribose) polymerase-1 (PARP-1). This accelerates the transfer of ADP-ribose groups to acceptor proteins by nicotinamide adenine dinucleotide $(NAD)^{+}$ metabolism.[28]

1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl -6,7-Isoquinolinediol reduced ROS production and intercellular Ca^{2+} accumulation in the present study, indicating that the neuroprotective effect of these two compounds may be related to their inhibition of ROS and intracellular Ca^{2+} production.

1,5-Isoquinolinediol prevented cognitive deficits and the aforementioned neurochemical alterations through oxidative stress-PARP pathway in the Hippocampus.[29] 1,2,3,4-tetrahydro-7,8-Isoquinolinediol

Table 3: 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-7-Isoquinolinol NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	9.43
OH aromatic C-OH	9.43
CH	4.29
CH_2	2.74, 2.64
CH 1-benzene	6.52
CH 1-benzene	6.70
CH 1-benzene	6.98
CH 1-benzene	7.07
CH 1-benzene	7.12
CH 1-benzene	6.70
CH 1-benzene	7.12
CH_2	2.77, 2.73
$CH_{3\ Methyl}$	2.26
CH_2	3.06, 2.81
C-13 NMR data	
C1	153.9
C2	155.7
C4	64.6
C5	47.5
C5	137.2
C6	127.3
C7	132.0
C8	114.3
C9	115.8
C10	108.1
C11	128.8
C12	130.2
C13	115.8
C14	130.2
C15	27.0
C16	42.5
C17	39.7

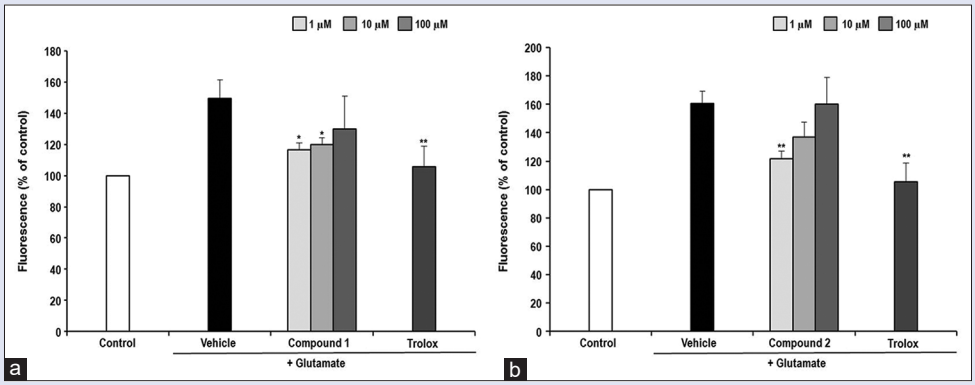


Figure 4: (a and b) Effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (2) on reactive oxygen species production in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P < 0.01$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus the glutamate-treated group

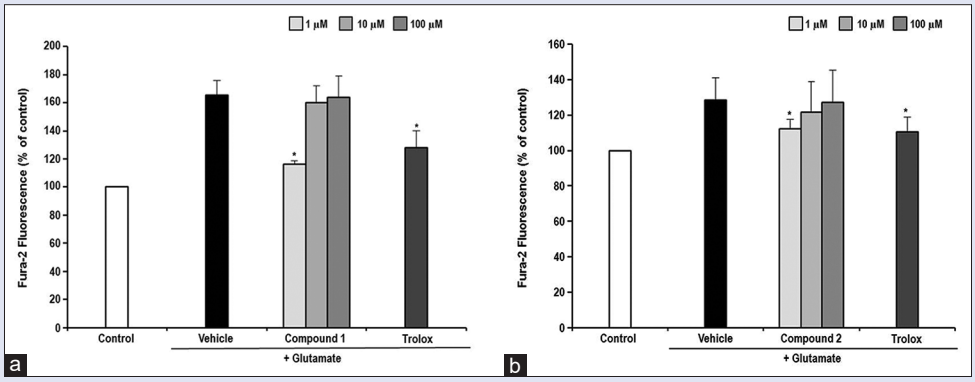


Figure 5: (a and b) Effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (2) on intracellular Ca^{2+} influx in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P < 0.01$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the glutamate-treated group

Table 4: 1-(3,4,5-trihydroxyphenyl)-ethanone NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	8.73
OH aromatic C-OH	9.48
OH aromatic C-OH	9.48
CH 1-benzene	6.84
CH 1-benzene	6.84
CH_3	2.50
C-13 NMR data	
C1	140.4
C2	146.1
C4	146.1
C5	132.1
C5	108.5
C6	108.5
C7	197.0
C8	26.6

Table 5: 1-(2,3,5,6-tetrahydroxyphenyl)-ethanone NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	13.78
OH aromatic C-OH	9.48
OH aromatic C-OH	13.78
OH aromatic C-OH	9.48
CH 1-benzene	6.35
CH_3	2.50
C-13 NMR Data	
C1	145.3
C2	141.2
C4	145.3
C5	141.2
C5	114.5
C6	110.8
C7	203.5
C8	32.8

is pheylethylamine-derived alkaloids, such as 1,5-isoquinolinediol. Therefore, we suggested that the effect of 1,2,3,4-tetrahydro-7,8-Isoquinolinediol is associated with oxidative stress-PARP overactivation cascade on the hippocampus

However, a lower concentration (1 mM) of 1,2,3,4-tetrahydro -7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1 -[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol showed a more potent neuroprotective effect than the higher concentration, meaning that the neuroprotective effects of these compounds were not simply due to an increased concentration.

Table 6: 3-(prop-1-enyl) benzene-1,2,4,5-tetrol NMR data

	Chemical shift (ppm)
H-1 NMR data	
OH aromatic C-OH	9.82
OH aromatic C-OH	9.48
OH aromatic C-OH	9.82
OH aromatic C-OH	9.48
CH 1-benzene	6.14
CH_3	1.80
C-13 NMR data	
C1	142.1
C2	140.9
C4	142.1
C5	140.9
C5	106.1
C6	80.9
C7	95.4
C8	4.8

Serotonin and norepinephrine are monoamine neurotransmitters, high doses of which induced neuronal cell death in a previous study.[30] The present data suggest that the monoamines, 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol may produce toxicity or failed to protect neuronal cells at a high concentration. In addition, the presence of alkyl or carboxylic groups in the aromatic ring of 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol slightly may increase the neuroprotective effect.

CONCLUSION

The embryo of *N. nucifera* seeds protects neuronal cells from glutamate-induced cell death. Six compounds were isolated from the embryo of *N. nucifera* seeds and the neuroprotective effect of 1,2,3,4-tetrahydro-7,8-Isoquinolinediol and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl)methyl]-2-methyl-6,7-Isoquinolinediol was much higher than that of the other compounds. These two compounds may exert a neuroprotective effect through the reduction of ROS levels and intracellular Ca^{2+} accumulation. Further studies will demonstrate the possible mechanism of the neuroprotective effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol and 1,2,3,4-tetrahydro -1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-isoquinolinediol.

Acknowledgements

We would like to express thanks to the staff and crew of the Central Laboratory of Kangwon National University NMR facility for their untiring efforts and perseverance.

Financial support and sponsorship

This work was supported by the National Research Foundation of Korea grant funded by the Korea government (Ministry of Science, ICT and Future Planning)(No. 2016R1A2B1011384). This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (No. 2018R1A6A1A03025582).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Crapper DR, DeBoni U. Brain aging and Alzheimer's disease. Can Psychiatr Assoc J 1978;23:229-33.
2. Swerdlow RH. Pathogenesis of Alzheimer's disease. Clin Interv Aging 2007;2:347-59.
3. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 1997;23:134-47.
4. Fukui M, Song JH, Choi J, Choi HJ, Zhu BT. Mechanism of glutamate-induced neurotoxicity in HT22 mouse hippocampal cells. Eur J Pharmacol 2009;617:1-11.
5. Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem Int 2004;45:583-95.
6. Chiang S, editor. Dictionary of Chinese Crude Drugs. Shanghai: Shanghai Scientific Technologic Publisher, New Medical College; 1978. p. 1806.
7. Sugimoto Y, Furutani S, Itoh A, Tanahashi T, Nakajima H Oshiro H, *et al.* Effects of extracts and neferine from the embryo of *N. nucifera* seeds on the central nervous system. Phytomedicine 2008;15:1117-24.
8. Itoh A, Saitoh T, Tani K, Uchigaki M, Sugimoto Y, Yamada J, *et al.* Bisbenzylisoquinoline Alkaloids from *Nelumbo nucifera*. Chem Pharm Bull (Tokyo) 2011;59:947-51.
9. Furukawa H. On the alkaloids of *Nelumbo nucifera* Gaertn. IX. Alkaloids of loti embryo. (2). structure of neferine, a new biscoclaurine alkaloid. Yakugaku Zasshi 1965;85:335-8.
10. Furukawa H. On the alkaloids of *N. nucifera* Gaertn. XII. Alkaloids of loti embryo. Yakugaku Zasshi 1966;86:75-7.
11. Nishibe S, Tsukamoto H, Kinoshita HS, Kitagawa S, Sakushima A. Sakushima A. Alkaloids from embryo of the seed of *N. nucifera*. J Nat Prod 1986;49:547-8.
12. Chen MS, Zhang JH, Wang JL, Gao L, Chen XX, Xiao JH. Anti-fibrotic effects of neferine on carbon tetrachloride-induced hepatic fibrosis in mice. Am J Chin Med 2015;43:231-40.
13. Yoon JS, Kim HM, Yadunandam AK, Kim NH, Jung HA, Choi JS, *et al.* Neferine isolated from *Nelumbo nucifera* enhances anti-cancer activities in Hep3B cells: Molecular mechanisms of cell cycle arrest, ER stress induced apoptosis and anti-angiogenic response. Phytomedicine 2013;20:1013-22.
14. Jung HA, Jin SE, Choi RJ, Kim DH, Kim YS, Ryu JH, *et al.* Anti-amnesic activity of neferine with antioxidant and anti-inflammatory capacities, as well as inhibition of ChEs and BACE1. Life Sci 2010;87:420-30.
15. Sugimoto Y, Furutani S, Nishimura K, Itoh A, Tanahashi T, Nakajima H, *et al.* Antidepressant-like effects of neferine in the forced swimming test involve the serotonin1A (5-HT1A) receptor in mice. Eur J Pharmacol 2010;634:62-7.
16. Wong VK, Wu AG, Wang JR, Liu L, Law BY. Neferine attenuates the protein level and toxicity of mutant huntingtin in PC-12 cells via induction of autophagy. Molecules 2015;20:3496-514.
17. Kim ES, Weon JB, Yun BR, Lee J, Eom MR, Oh KH, *et al.* Cognitive enhancing and neuroprotective effect of the embryo of the *Nelumbo nucifera* seed. Evid Based Complement Alternat Med 2014;2014. doi: 10.1155/2014/869831.
18. Liu J, Li L, Suo WZ. HT22 hippocampal neuronal cell line possesses functional cholinergic properties. Life Sci 2009;84:267-71.
19. Rajagopalan P, Pachter IJ. Tris [Tetrahydroisoquinoline] Compounds U.S. Patent. Vol. 4. Washington, DC: U.S. Patent and Trademark Office; 1978. p. 107-65.
20. Hawkins KM, Smolke CD. Production of benzylisoquinoline alkaloids in Saccharomyces cerevisiae. Nat Chem Biol 2008;4:564-73.
21. Feng S, Jiang J, Hu P, Zhang JY, Liu T, Zhao Q, *et al.* A phase I study on pharmacokinetics and pharmacodynamics of higenamine in healthy Chinese subjects. Acta Pharmacol Sin 2012;33:1353-8.
22. Robert M. Handbook of Hydroxyacetophenones. Berlin: Springer-Science+Business Media, B.V; 1997.
23. Rau T, Ziemniak J, Poulsen D. The neuroprotective potential of low-dose methamphetamine in preclinical models of stroke and traumatic brain injury. Prog Neuropsychopharmacol Biol Psychiatry 2016;64:231-6.
24. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, *et al.* Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. Neuron 1995;15:961-73.
25. Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron

1989;2:1547-58.

26. Randall RD, Thayer SA. Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. J Neurosci 1992;12:1882-95.
27. Reynolds IJ, Hastings TG. Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. J Neurosci 1995;15:3318-27.
28. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 2013;12:931-47.
29. Pant R, Jangra A, Kwatra M, Singh T, Kushwah P, Bezbaruah BK, *et al*. Cognitive deficits induced by combined exposure of stress and alcohol mediated through oxidative stress-PARP pathway in the hippocampus. Neurosci Lett 2017;653:208-14.
30. Apel K, Hirt H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004;55:373-99.