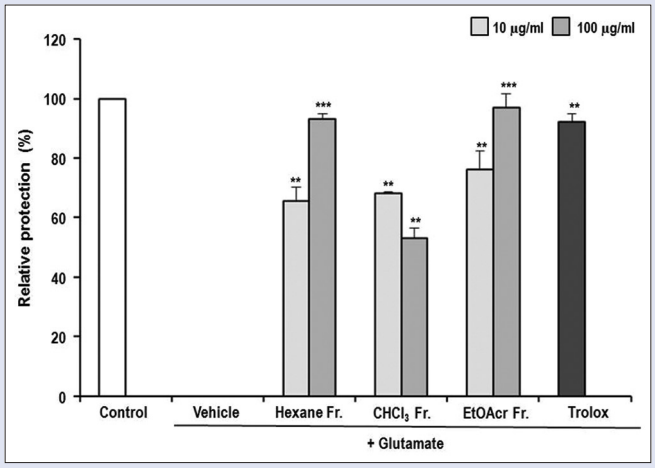

Figure 2: Neuroprotective effect of the fractions (hexane, chloroform, ethyl acetate, butanol) of *Nelumbo nucifera* seeds. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P < 0.01$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P 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

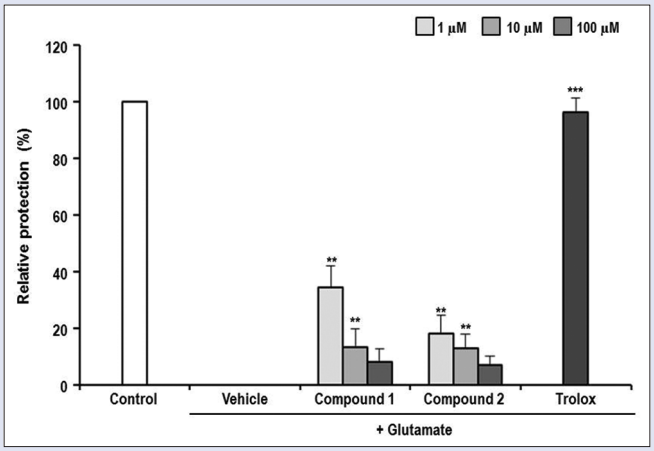

Figure 3: Neuroprotective effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl -6,7-Isoquinolinediol (2) on glutamate-induced cell death in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments.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

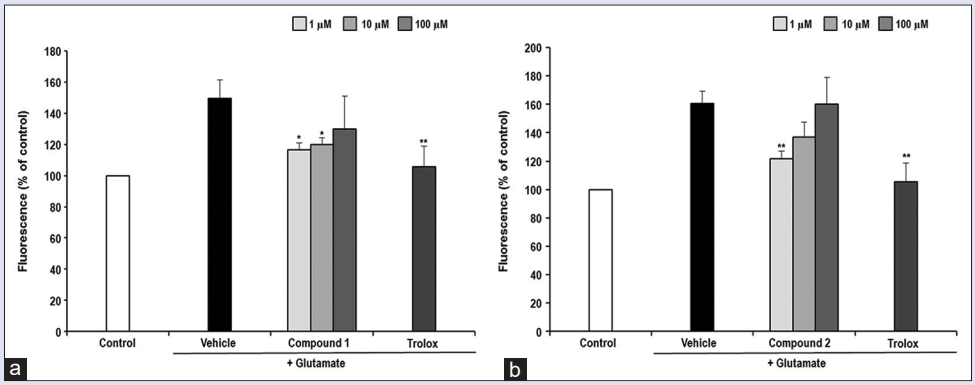

Figure 4: (a and b) Effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (2) on reactive oxygen species production in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments.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

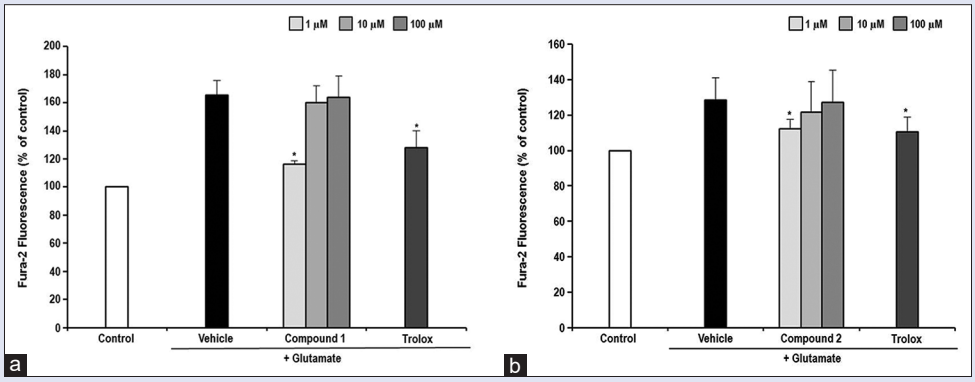

Figure 5: (a and b) Effect of 1,2,3,4-tetrahydro-7,8-isoquinolinediol (1) and 1,2,3,4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-2-methyl-6,7-Isoquinolinediol (2) on intracellular Ca^{2+} influx in HT-22 cells. Data represent the mean ± standard error of the mean of three independent experiments. $^{\#\#}P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

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Conflicts of interest

There are no conflicts of interest.

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