

Effects of *Coriandrum sativum* extracts on glucose/serum deprivation-induced neuronal cell death

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Abstract

Objective: This study was planned to investigate whether *Coriandrum sativum* (*C. sativum*) is capable of protecting neurons against glucose/serum deprivation (GSD)-induced cytotoxicity.

Material and Methods: The PC12 cells were cultivated for 24 h in standard media (high-glucose DMEM containing Fetal Bovine Serum) or for 6 h in GSD condition (glucose-free DMEM, without serum) in the absence or presence of various concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml) of hydro-alcoholic extract (HAE), water fraction (WF), ethyl acetate fraction (EAF) or N-butanol fraction (NBF) of this plant. At the end of the treatments, the cell viability was determined using MTT assay.

Results: With the exception of 1.6 mg/ml of EAF or NBF which decreased cell survival, the HAE and its fractions exhibited no cytotoxicity under standard condition. Exposure of the cells to GSD condition showed 52% decrease in the viability. In this condition, the HAE, EAF and NBF not only failed to increase cell viability but also increased the toxicity. On the other hand, WF at 0.4, 0.8 and 1.6 mg/ml significantly attenuated the GSD-induced decrease in cell survival.

Conclusion: The present study revealed that *C. sativum* bearing water-soluble compound(s) could induce neuroprotective activity. Also, we showed that some constituents from this plant may serve as cytotoxic agents under stressful conditions like hypoglycemia and serum limitation.

Keywords: *Coriandrum sativum*; Glucose/serum deprivation; Neuroprotective; PC12

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Introduction

Cerebral ischemia is still one of the most leading causes of death and long-term disability in the world. Restriction of blood flow to the ischemic area results in insufficient oxygen and glucose delivery to that area and finally leads to cell death. Mechanisms of cell damage include apoptosis, acidosis, inflammation, excitotoxicity and oxidative stress (Bramlett and Dietrich, 2004; Doyle et al., 2008). The brain is especially sensitive to oxidative stress due to its high rate of oxidative metabolic activity (Maier and Chan, 2002) and having relatively low levels of endogenous antioxidants (Doyle et al., 2008). Therefore, a promising approach to neuroprotection from ischemia is the use of antioxidants (Ochiaia et al., 2004).

Cultured neural cells can undergo apoptosis in response to component stimuli of ischemia, such as hypoxia, serum and nutrient deprivation, and metabolic stress. Glucose/serum deprivation (GSD) has served as an excellent in vitro model for the understanding of the molecular mechanisms of neuronal damage during brain ischemia and for the development of neuroprotective drugs against ischemia-induced brain injury (Chu et al., 2008; Hillion et al., 2005; Mousavi et al., 2010).

At present, there are very few medications for the management of cerebral ischemia, with limited success. Therefore, the search for new therapeutics has continued. Medicinal plants have always been a good source to find new remedies for human health problems.

Coriandrum sativum (Coriandre), an annual herb belonging to the Apiaceae family, has been considered as a medicinal plant for a long time. Coriandre is used in folk medicine as a stomachic, carminative, stimulant, nerve sedative and antiepileptic (Zargari, 1995). This plant has also been reported to have a number of medicinal attributes including hypolipidemic, antidiabetic, and hepatoprotective effects

(Dhanapakiam et al., 2008; Eidi et al., 2009; Samojlik et al., 2010). Experimental studies have also revealed strong antioxidant activity of *C. sativum* that is superior to known antioxidants like ascorbic acid (Guerra et al., 2005; Hashim et al., 2005; Misharina and Samusenko, 2008; Samojlik et al., 2010; Satyanarayana et al., 2004; Sultana et al., 2010; Wangenstein et al., 2004). Therefore, this study was carried out to investigate whether *C. sativum* is capable of protecting neurons against glucose/serum deprivation (GSD)-induced cytotoxicity, an excellent in vitro model which simulates neuronal damage during brain ischemia (Hillion et al., 2005).

Materials and Methods

Drugs and chemicals

Dimethyl sulfoxide (DMSO), penicillin-streptomycin and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-Diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma (USA). Dulbeccos Modified Eagles Medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from GIBCO (USA).

Plant and extracts

The aerial parts (leaves, stems, twigs) of *C. sativum* were collected from Neyshabur area, (Razavi Khorasan state, Iran). The identity of the plant was confirmed and for future reference a voucher specimen (10068) was deposited at the herbarium of School of Pharmacy (Mashhad University of Medical Sciences, Iran). The plant materials were cleaned dried at room temperature and ground to fine powder with a blender. The hydro-alcoholic extract (HAE) was prepared by the extraction of the plant materials in 70% ethanol for 48 h using a Soxhlet apparatus. The HAE was then dried on a water bath and the yield (33% w/w) was dissolved in DMSO.

For preparation of fractions, a part of dried HAE was suspended in distilled water and then transferred to a separator funnel.

Through solvent-solvent extraction, it was sequentially fractionated with ethyl acetate and N-butanol. The fractions of N-butanol (NBF) and ethyl acetate (EAF) were separated to obtain water fraction (WF). The resulting fractions were evaporated to dryness and then dissolved in DMSO and saline for EAF or NBF and WF, respectively.

Cell culture and treatment

The PC12 cells, a rat pheochromocytoma-derived cell line, were cultivated in high-glucose DMEM (4.5g/l) supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin in humidified atmosphere of 5% CO₂ and 95% air at 37 °C. The cells at subconfluent stage were harvested from flask by treatment with trypsin and an aliquot of 200 µl of cell suspension (5×10^4 cells/ml) was transferred to each well of 96-well tissue culture plate. The cells were cultivated for 48 h at CO₂ incubator. Then the cells were treated with the extracts under two conditions of cell culture; standard and GSD conditions. In standard condition, the high-glucose DMEM was changed by fresh one containing 10% FBS and varying concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml) of HAE, WF, EAF or NBF. Working solutions of all extracts were made up in high-glucose DMEM. In the GSD condition, the high-glucose medium was replaced by glucose-free DMEM (without FBS) containing the extracts. In this model, the working solutions were made up in glucose-free DMEM. Duration of treatment was 24 and 6 h for the standard and GSD conditions, respectively.

Cell viability assay

At the end of the treatment, the cell viability was determined using MTT colorimetric assay as previously described (Tavakkol Afshari *et al.*, 2006). Briefly, the MTT solution was added to each well to make final concentration of 0.5 mg/ml and the reaction mixture incubated for 2 h.

Then, the mixture was removed and the resulting formazan dissolved by adding 200 µl DMSO to each well. The optical density of formazan dye was read at 540 nm.

Statistical analysis

All data were expressed as mean±SEM. One way ANOVA followed by Tukey's post hoc test for multiple comparisons were used for statistical evaluation. Statistical significance was accepted at $p < 0.05$.

Results

Effect of *C. sativum* on cell viability in standard condition

When the HAE of *C. sativum* and its fractions were evaluated for *in vitro* cytotoxicity, it was found that after 24 h only high concentration of EAF and NBF caused neuronal toxicity (Figure 1). When compared with untreated cells, the EAF at 1.6 mg/ml decreased the surviving cells to 12.5 ± 0.2 % ($p < 0.001$), which was lowest among all treatments. Also, the presence of NBF at 1.6 mg/ml in the culture medium led to a significant decrease in survival of PC12 cells (56.6 ± 7 %, $p < 0.05$). The HAE and WF exhibited no cytotoxicity against this cell line even at the highest concentration.

Effect of *C. sativum* on cell viability in GSD condition

As shown in Figure 2, exposure of the PC12 cells to GSD condition showed 52% decrease in cell viability. The HAE at all concentrations used, not only failed to increase cell survival but even further decreased the viability. The percent of relative cell viability was 22 ± 4 , 9 ± 1 , 9 ± 0.6 , 9.3 ± 0.4 and 9.4 ± 0.2 % after treatment with 0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml of HAE, respectively. Statistically, these levels of cell survival were significantly ($p < 0.001$) lower than those of untreated cells cultured in the standard or in GSD conditions.

Similarly, all concentrations of EAF and NBF (with the exception of 1.6 mg/ml)

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induced significant decrease in viability of PC12 cells. Incubation of the cells with 0.1, 0.2, 0.4 and 0.8 mg/ml of EAF decreased the viability to 7.3 ± 0.6 , 9 ± 0.6 , 10 ± 0.4 and $29\pm 2.4\%$, respectively. Also, when the cells were exposed to 0.1, 0.2, 0.4 and 0.8 mg/ml of NBF, the level of cell survival decreased to 10.2 ± 1.1 , 12.6 ± 1.2 , 15.5 ± 0.4 and $28.3\pm 2.4\%$, respectively.

At concentration of 1.6 mg/ml, the EAF or NBF had no significant effect on viability of PC12 cells.

On the other hand, WF at high concentrations significantly attenuated the GSD-induced decrease in cell viability. About 65% of the PC12 cells incubated with 0.8 or 1.6 mg/ml of WF was found to be alive. This level of viability was significantly ($p < 0.05$) higher than that observed in untreated cells. Effect of 0.1 mg/ml ($47\pm 2.2\%$), 0.2 mg/ml ($56\pm 2.7\%$) and 0.4 mg/ml ($61\pm 2\%$) of WF on cell survival remained statistically insignificant.

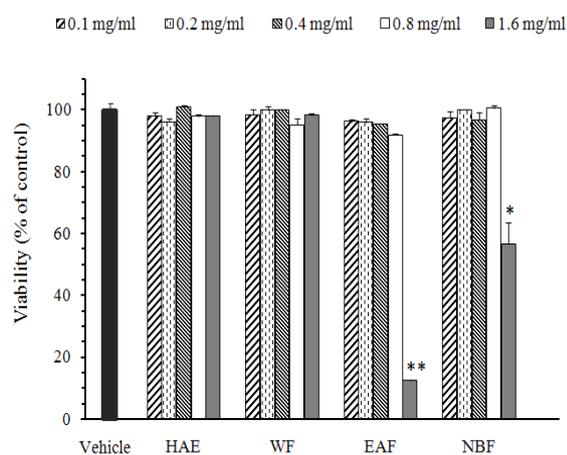


Figure 1. Effect of *C. sativum* on viability of PC12 cells cultured in the standard condition. The cells were cultivated for 24 h in high-glucose DMEM supplemented with 10% Fetal Bovine Serum and containing vehicle or various concentrations of the extracts. The bars show percentage of cell viability as compared with untreated cells (vehicle). Results represent mean \pm SEM of one experiment performed in triplicate. * $p < 0.001$ versus vehicle; ** $p < 0.01$ versus vehicle. HAE: hydro-alcoholic extract; WF: water fraction; EAF: ethyl acetate fraction; NBF; N-butanol fraction.

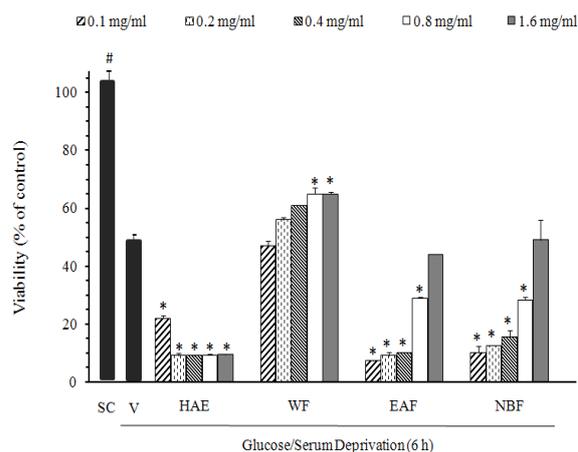


Figure 2. Effect of *C. sativum* on viability of PC12 cells cultured in glucose/serum deprivation condition. The cells were cultivated for 6 h in glucose-free DMEM without Fetal Bovine Serum but containing the extracts or vehicle. The bars show percentage of cell viability as compared with untreated cells cultured in the standard condition. Results represent mean \pm SEM of two separate experiments performed in triplicate. * $p < 0.05$ versus V; # $p < 0.001$ versus all other incubations. SC: standard condition; V: vehicle; HAE: hydro-alcoholic extract; WF: water fraction; EAF: ethyl acetate fraction; NBF; N-butanol fraction.

Discussion

Oxidative stress is the basis for many neurological and neurodegenerative disorders (Amantea et al., 2009). Nowadays attention has increased to use antioxidants for protection of neurons against oxidative damage (Gilgun-Sherki et al., 2002). Previous studies demonstrated that *C. sativum* had strong antioxidant activity which was even superior to known antioxidants like ascorbic acid (Guerra et al., 2005; Hashim et al., 2005; Misharina and Samusenko, 2008; Samojlik et al., 2010; Satyanarayana et al., 2004; Sultana et al., 2010; Wangenstein et al., 2004). In the present study, we used GSD-induced neurotoxicity to partially mimic the pathological process of cerebral ischemia and to investigate the possible

neuroprotective effect of *C. sativum*. The results showed that the viability of PC12 cells was significantly decreased in GSD condition. This was expected to happen and indicated that our culture condition was well optimized. Under this condition, the HAE of *C. sativum* not only failed to increase cell viability but even further decreased that which was unexpected. In order to obtain better insight into the nature of compounds responsible for this activity, three fractions were prepared from HAE: (1) The WF with water-soluble constituents; (2) The EAF with compounds of intermediate polarity; (3) The NBF with lipid-soluble agents (Seidel, 2006).

Interestingly, positive results were obtained when the PC12 cells were treated with WF. This attenuated the GSD-induced decrease in cell viability and had no cytotoxic effect on the cells cultured in the standard condition either. On the other hand, both EAF and NBF have similar negative effect as HAE on the viability of PC12 cells cultured in the GSD condition. We know that natural antioxidants have different solubilities: water-soluble ascorbic acid, phenolic compounds, glutathione and urate; lipid-soluble tocopherols and carotenoids, and intermediary-soluble flavonoids and hydroxycinnamic acids (Eastwood, 1999; Podsedek, 2007). Our results regarding WF, raises this possibility that water-soluble antioxidants are involved in the neuroprotective effect of *C. sativum*. This view is supported by the findings of Satyanarayana et al. (2004) who showed aqueous extract of *C. sativum* had strong antioxidant activity. However, other studies reported that essential oil and EAF of this plant can also exert the same activity (Misharina and Samusenko, 2008; Samojlik et al., 2010; Wangenstein et al., 2004). Therefore, the nature of component(s) responsible for the neuroprotective effect of *C. sativum* is still an open question.

Under standard condition, we observed that only at high concentration could EAF and NBF decrease surviving of PC12 cells.

This observation in conjunction with our findings about the effects of these fractions in the GSD condition encouraged us to speculate that in standard condition the PC12 cells are able to resist stressful properties of the extracts. However, when the cells are irritated with GSD, they lost a part of this ability.

In summary, the present study revealed that some constituents of *C. sativum* may serve as cytotoxic agents under stressful conditions like hypoglycemia and serum limitation. On the other hand, this plant bears water-soluble compound (s) which induce neuroprotection and could be considered for treatment of common neurological disorders. Further isolation and identification of the active compound (s) in WF are recommended.

Acknowledgments

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