

Effects of Some Lamiaceae Species on NO Production and Cell Injury in Hydrogen Peroxide-induced Stress

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Abstract

Nitric oxide (NO) is a key mediator that plays an important role in pathogenesis of various chronic diseases like Alzheimer's disease and Parkinson's disease. Additionally, there is a great attitude for finding natural compounds, which could control and inhibit NO production in pathological conditions. Therefore, we were encouraged to investigate the effects of some Lamiaceae species on NO production and cell injury during oxidative stress in PC12 cells. In this study, cell death determined by MTT assay and NO levels were evaluated using Griess assay. PC12 cells were exposed to total metanolic extracts of three *Scutellaria* and one *Nepeta* species. The results revealed that *Nepeta laxiflora* (*N. laxiflora*) could protect PC12 cells from hydrogen peroxide-induced oxidative stress and all of the plants inhibited NO production in that condition except *Scutellaria tournefortii* (*Sc. tournefortii*). In addition, *Scutellaria multicaulis* (*Sc. multicaulis*) was meanwhile subjected to fractionation using different organic solvents. The dichloromethane and ethyl acetate fractions of *Sc. multicaulis* could protect PC12 cells from oxidative stress injury. However, NO production was restrained by the hexane and dichloromethane fractions. Considering the results, *N. laxiflora*, *Scutellaria nepetifolia* (*Sc. nepetifolia*), and *Sc. multicaulis* are good candidates for further investigations in neuroprotection and anti-inflammation studies.

Keywords: *Scutellaria*; *Nepeta*; PC12 cells; Griess assay; Nitric oxide; Oxidative stress.

Introduction

Nitric oxide (NO) is a gaseous mediator produced by oxidation of L-arginine. Remarkably, three types of nitric oxide synthase (NOS) enzymes have been identified and two of them, neuronal and endothelial

isoforms (nNOS, eNOS), are constitutive Ca²⁺-dependent and act in physiological conditions. On the other hand, the inducible form (iNOS) can produce NO increasingly in pathological cellular environments (1). Classically, NO signaling was attributed to cyclic guanosine monophosphate (cGMP) especially in endothelial cells. However, recent researches reveal the effects of NO on other pathways. So it can be stimulated by various factors

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and in conditions, such as nitrosative stress it promotes apoptosis either dependent or independent on mitochondria (2).

Additionally, oxidative/nitrosative stress (ONS) is defined as an imbalance between pro-oxidants (reactive oxygen and nitrogen species) and antioxidants in favor of the former. Over the last decades, numerous studies have shown the key role of ONS in variety of pathologies, including chronic degenerative disease of the brain (3). Moreover, the production of nitric oxide (NO) and several pro-inflammatory factors increase in some pathological conditions and promote neuroinflammation. Notably, the neuroinflammation has a central position in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (4). Furthermore, NO by changing the redox state of cell microenvironments and DNA modification or mediating angiogenesis can include cancer progression (1). Besides, in asthma pathogenesis, recent studies support the key roles of NO and reactive nitrogen species (RNS) (5).

Considering foregoing matters, there is a huge interest in discovery of drugs or lead compounds, which could control and inhibit NO production especially in pathological conditions. Besides, some of the plant extracts are good candidates as a source for discovery of such compounds due to their antioxidant and anti-inflammatory properties. Usually these extracts have main compounds related to phenolic structures such as flavonoids (6, 7).

Notably, Lamiaceae is one of the largest flowering plant families that has 47 genera; and 371 species, sub-species, and varieties in Iran. Moreover, the plants in Lamiaceae have a wide range of biological activities and diversity in phytochemical classes such as terpenes, flavonoids, and phenolic acids (8, 9). According to a review, Lamiaceae was the most appeared plant family in Iran ethnopharmacological studies (9).

In addition, genera *Nepeta* and *Scutellaria* from Lamiaceae, have shown promising effects in antioxidant and anti-inflammation bioassays, and both of them are rich sources of phenolic and terpene compounds (10, 11).

In the present study, we investigated the

inhibitory effect of total extracts and fractions of three plants from *Scutellaria* genus (two of them are endemic to Iran) and one Iran endemic plant from *Nepeta* genus, in PC12 cells, stressed by hydrogen peroxide.

Experimental

Chemicals

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA solution, penicillin/streptomycin 100 units, dimethylsulfoxide (DMSO), and all other fine chemicals were obtained from Merck, Germany. 3-(4,5 dimethylthiazole-2-yl)-2,5-dimethyl tetrazolium bromide (MTT) and trypan blue were purchased from Sigma (St Louis, MO).

Plant materials

Three endemic plants including *Scutellaria nepetifolia* Benth., *Sc. multicaulis* subsp. *multicaulis* Boiss., and *Nepeta laxiflora* Benth. were collected in mid July 2016 from Hamedan province and another plant *Sc. tournefortii* Benth. was collected in May 2016 from Golestan province of Iran. All plants were identified by botanists at Tehran University of Medical Sciences (TUMC) and Shahid Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen of each species is deposited at respective herbarium.

Preparation of extracts

Aerial part of each plant was shade dried, powdered, and macerated with methanol in 1 to 10 proportion, respectively. Maceration lasted for 72 h and a fresh solvent replaced the extract every 24 h. For fractionation of the selected plant, maceration was carried out with the same process except that n-hexane, dichloromethane, ethyl acetate, and methanol were used as solvents, respectively. All extracts and fractions were collected and concentrated by rotary evaporator Heidolph 4000 (Schwabach, Germany) at room temperature, to remove all solvent residuals.

Cell culture and treatment

PC12 (rat pheochromocytoma) cells

obtained from Pasteur Institute (Tehran, Iran), were grown in DMEM enriched by 10% FBS, supplemented with 100 unit/mL penicillin and 100 mg/mL streptomycin and maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO₂.

Plant extracts and fractions were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions. The stock solutions and hydrogen peroxide (H₂O₂) were diluted with DMEM to gain interested concentrations prior to use. All of the experiments involving exposure to H₂O₂ and measurement of NO were performed in serum-free DMEM to avoid rapid H₂O₂ degradation by antioxidants and interference with Griess assay by FBS (12, 13).

Cell Viability Assay

The effect of extracts on cell viability was assessed by colorimetric method with MTT salt (14). PC12 cells were seeded in 96-well plates at a density of 35000 cells/well and incubated for 24 h. Then the medium was replaced with fresh medium containing different concentrations of each extract (25-300 µg/mL) and incubated for 24 h. Negative control wells were treated with 1% (v/v) DMSO in equal volume of medium. In the next step, the supernatant was replaced with the fresh medium, containing MTT salt (0.5 mg/mL), and incubated for 24 h. In the last step, the culture supernatant was aspirated and the formazan crystals were dissolved in DMSO. ELISA plate reader from Fisher Scientific Company (Ontario, USA) measured the absorbance of wells at 570 nm. The results presented the percentage of mean absorbance of negative control wells, considered as 100% viability.

The above procedure was repeated for the fractions of the selected plant in 50 to 200 µg/mL concentration range.

Inducing oxidative stress

To determine the suitable concentration of H₂O₂, PC12 cells were seeded in 96-well plates at a density of 35000 cells/well. After 24 h incubation, the medium was freshened and all the cells were treated by 1% (v/v) DMSO and incubated for another 24 h. Then the medium was replaced by FBS-free DMEM and

the cells were treated with H₂O₂ (1–2.6 mM) for 24 h and the cell viability was detected by the MTT method as described previously. The concentration that produced about 50% viability (EC₅₀) was selected for the following tests.

Cytoprotection by extracts and fractions as pretreatment

For assessing extracts and fractions effect on cell viability in stressed PC12, after 24 h incubation of cells in 96-well plate (35000 cells/well), the medium was replaced and the cells were treated by DMEM-FBS10% contains extracts (25-300 µg/mL) and fractions of selected plant (50-200 µg/mL). Two groups of negative control wells were treated with 1% (v/v) DMSO in equal volume of medium. After 24 h incubation, the medium replaced by FBS free DMEM contains H₂O₂ (1.5 mM), except for one group of negative control (control versus stressed-control group). Twenty-four hours later, cell viability was detected by the MTT method as described previously.

Griess assay

A standard procedure was used to determine NO production in stressed PC12 cells (15). Briefly after seeding cells in 96-well plate and 24 h treatment with H₂O₂, as described above, equal volume (100 µL) of Griess reagent [1:1 mixture (v/v) of 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% H₃PO₄] and supernatant of each well was mixed and incubated for 15 min in dark place at room temperature. All extracts and fractions were employed in a process similar to that was described in the previous section. Subsequently, the absorbance of the mixture was determined at 540 nm with ELISA plate reader from Fisher Scientific Company (Ontario, USA).

Statistical analysis

The data were presented as the mean ± SEM. To compare the group means, one-way analysis of variance (ANOVA), followed by Dunnett's and Sidak's posttests were used. All Statistical analysis were carried out by GraphPad Prism software version 6.01 from GraphPad Software Inc. (San Diego, CA, USA).

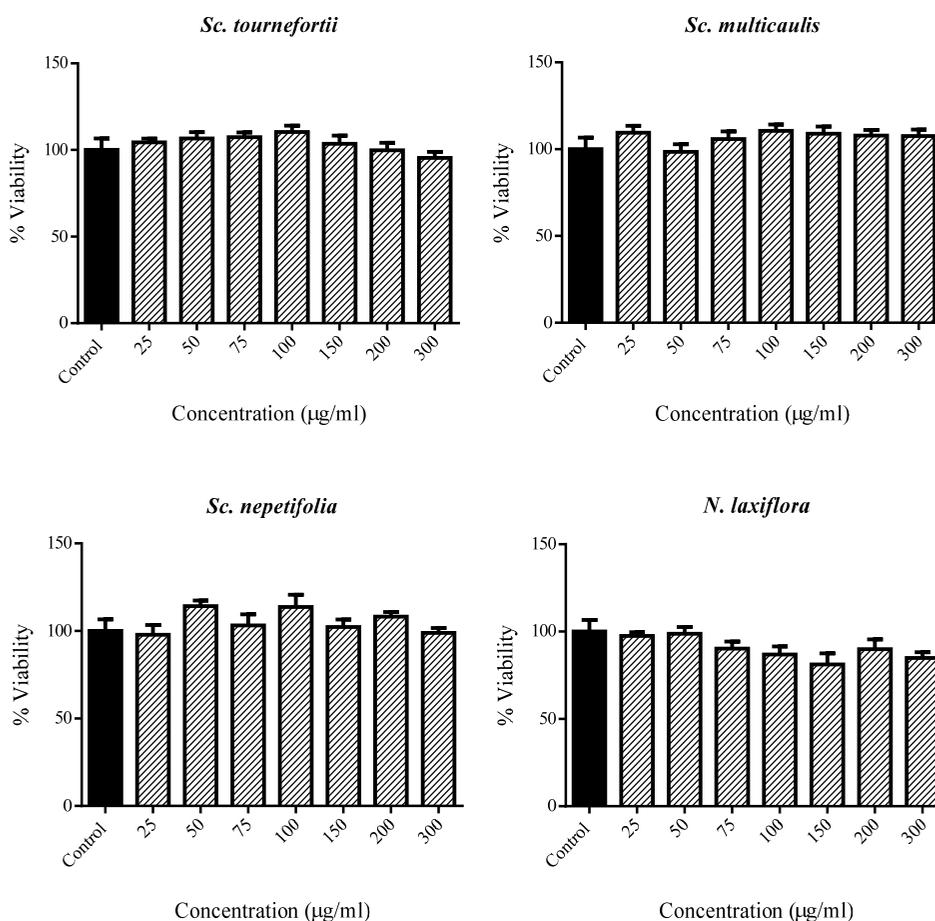


Figure 1. The viability percent of PC12 cells that treated with various concentration of tested plants for 24 h. Data were expressed as percentage of control group mean absorbance (Viability%) and represent as mean \pm SEM (n = 6).

Results

Effect of extracts on cell viability in PC12 cells

The cytotoxicity of the extracts in 50-100 µg/mL concentrations were assessed by MTT method in PC12 cells. There is no cytotoxicity observed for any of extracts of *Sc. nepetifolia*, *Sc. multicaulis*, *Sc. tournefortii*, and *N. laxiflora* in the tested range (Figure 1).

EC₅₀ of H₂O₂

The data for viability percent of cells in presence of H₂O₂ (1-2.6 mM) were used for fitting a nonlinear regression. According to the result, EC₅₀ of H₂O₂ calculated 1.540 mM

with 95% confidence interval of 1.507 to 1.574 mM. Therefore, the concentration of 1.5 mM was selected for inducing the oxidative stress in PC12 cells.

Cytoprotective effect of extracts in H₂O₂-induced oxidative stress

The PC12 cells viability was assessed in oxidative stress condition in the presence of the tested plants' total extracts. The result (Figure 2) showed the statistically significant difference between the control and stressed-control group viability percent ($p < 0.001$), and the viability percent of stressed-control group was 69%. Hereon, the oxidative stress was effectively implied. Among the extracts, only

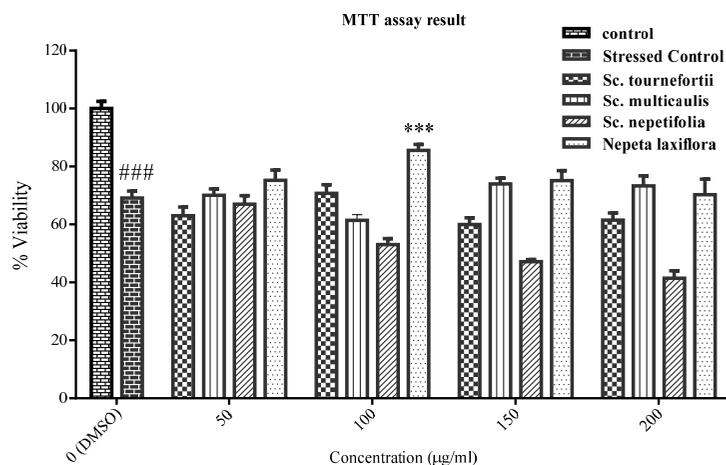


Figure 2. The viability percent of PC12 cells that pre-treated with various concentration of tested plants for 24 h and afterward treated with H₂O₂ 1.5 mM for another 24 h. Data were expressed as percentage of control group mean absorbance (Viability%) and represent as mean ± SEM (n = 6). ### and ****p* < 0.001 compared to control and stressed-control group, respectively.

total extract of *Nepeta laxiflora* at 100 µg/mL concentration could protect the cells against oxidative stress, significantly (*p* < 0.001).

The extracts effects on NO production

The inhibitory effect of the tested plants was examined by Griess assay, after pre-treatment of PC12 cells with the total extracts for 24 h and subsequently treatment with H₂O₂

1.5 mM for another 24 h. According to data (Figure 3), NO production was increased in the stressed-control group compared to control cells, significantly (*p* < 0.001). The total extract of *Sc. tournefortii* showed no inhibitory effect on NO producing in any concentration and interestingly showed a significant positive effect in 100 µg/mL (*p* < 0.05). As observed in Figure 3, both *Sc. nepetifolia* and *N. laxiflora*

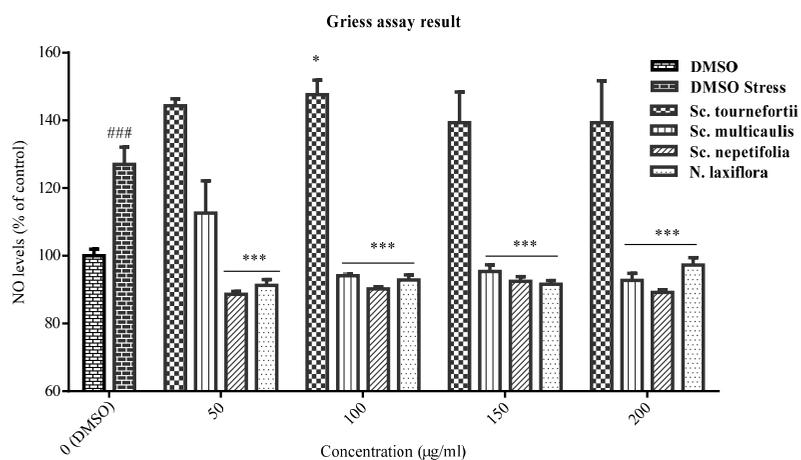


Figure 3. The effect of total extracts on NO production was assessed in PC12 cells that treated with H₂O₂ 1.5 mM for 24 h. Data were expressed as percentage of control group mean absorbance (% of control) and represent as mean ± SEM (n = 6). ### and ****p* < 0.001 (**p* < 0.05) compared to control and stressed-control group, respectively.

Table 1. The calculated IC₅₀ for each *Sc. multicaulis* of fractions in PC12 cells.

Fraction	IC ₅₀ (µg/mL)	R square
n-Hexane	105.5	0.97
Dichloromethane	n/a	n/a
Ethyl acetate	106.3	0.88
Methanol	n/a	n/a

total extracts inhibit NO production in all concentrations significantly and *Sc. multicaulis* represented similar effect in all concentrations except 50 µg/mL ($p < 0.001$).

Effect of selected plant fractions on cell viability in PC12 cells

Cytotoxicity of fractions of *Sc. multicaulis* was determined in 50-200 µg/mL range with a method similar to that, used for the total extracts. As expressed in Table 1, the half maximal inhibitory concentration (IC₅₀) of n-hexane and ethyl acetate fractions were calculated as 105.5 and 106.3 µg/mL, respectively. Hence, methanol and dichloromethane fractions did not give under 50% viability in any concentration, IC₅₀ for these fractions were estimated higher than 200 µg/mL (Figure 4). Therefore, the concentrations under 100 µg/mL were selected

for oxidative stress test.

Cytoprotective effect of *Sc. multicaulis* fractions in H₂O₂-induced oxidative stress

The PC12 cells viability was assessed in oxidative stress condition after pretreatment with tested fractions (25-100 µg/mL) for 24 h. As expected, the oxidative stress was induced successfully and the stressed-control group viability percent (65%) differed from the control group significantly ($p < 0.001$). In addition, as seen in Figure 5, the hexane fraction decreased the cell viability in 25 and 100 µg/mL ($p < 0.01$ and $p < 0.001$, respectively). The dichloromethane fraction showed protective effect in 50 and 100 µg/mL ($p < 0.05$ and $p < 0.01$, respectively) and also showed toxic effect only in 25 µg/mL.

Besides, the ethyl acetate fraction had a

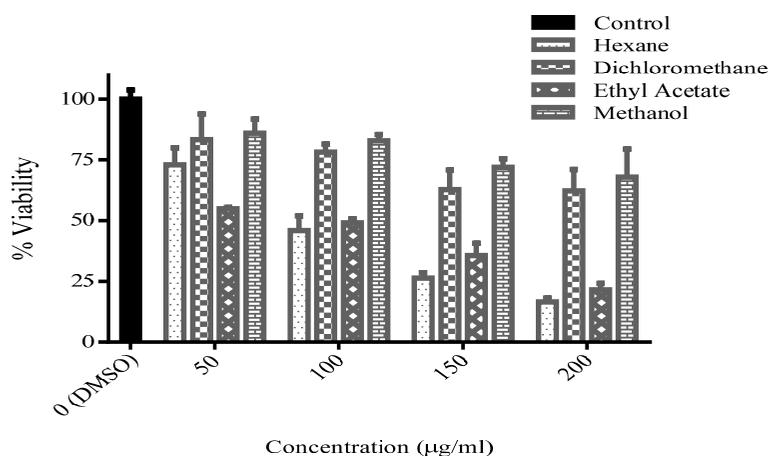


Figure 4. The viability percent of PC12 cells that treated with various concentration of *Sc. multicaulis* fractions for 24 h. Data were expressed as percentage of control group mean absorbance (Viability%) and represent as mean ± SEM (n = 6).

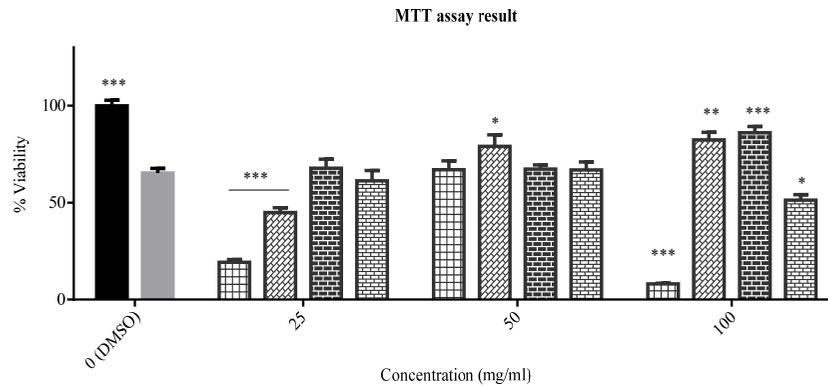


Figure 5. The viability percent of PC12 cells that pre-treated with *Sc. multicaulis* fractions for 24 h and afterward treated with H₂O₂ 1.5 mM for another 24 h. Data were expressed as percentage of control group mean absorbance (Viability%) and represent as mean ± SEM (n = 6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 compared to stressed-control group. Hex, the n-hexane fraction; DCM, the dichloromethane fraction; EtOAc, the ethyl acetate fraction; MeOH, the methanol fraction.

protective effect in 100 µg/mL significantly (*p* < 0.001) and the methanol fraction promoted cytotoxic effect in 100 µg/mL significantly (*p* < 0.05).

The extracts effects on NO production

After pre-treatment of PC12 cells with *Sc. multicaulis* fractions and subsequently treatment with H₂O₂ 1.5 mM, the inhibitory effect on NO was examined by Griess assay. Result, showed in Figure 6, proved the strong inhibitory effect of the hexane and

dichloromethane fractions of *Sc. multicaulis* at 25 and 50 µg/mL (*p* < 0.001). The hexane fraction had this effect in 100 µg/mL, too (*p* < 0.001). On the other side, none of the ethyl acetate or methanol fractions could inhibit NO production in stressed PC12 cells.

Discussion

As concerned previously, oxidative stress plays a crucial role in the pathogenesis of chronic diseases and inflammation

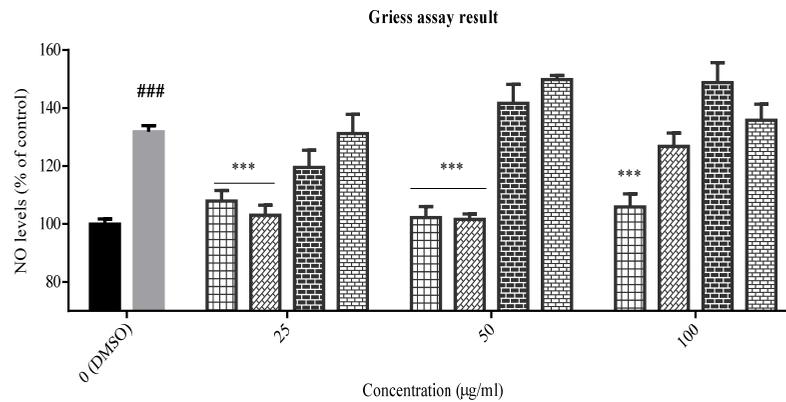


Figure 6. The effect of *Sc. multicaulis* fractions on NO production was assessed in PC12 cells that treated with H₂O₂ 1.5 mM for 24 h. Data were expressed as percentage of control group mean absorbance (% of control) and represent as mean ± SEM (n = 6). ### and ****p* < 0.001 compared to control and stressed-control group, respectively. Hex, the n-hexane fraction; DCM, the dichloromethane fraction; EtOAc, the ethyl acetate fraction; MeOH, the methanol fraction.

including neurodegenerative disorders like Alzheimer's disease (16). As a result, finding natural products with antioxidant and anti-inflammation properties is an interesting subject to recent researches. Notably, the Lamiaceae family has a great potential in drug discovery and some of its genera like *Nepeta* and *Scutellaria* are known for their remarkable antioxidant, anti-inflammatory, and neuroprotective effects (8, 11). The *Scutellaria* species have memory-enhancing, immunostimulant, anti-inflammatory, antiepileptic, and hepatoprotective properties, and were used in Chinese and Korean traditional medicine. In addition, the *Scutellaria* genus has 10 endemic species in Iran (8, 17). On the other hand, the *Nepeta* species are well-known for their antitumor, anti-inflammatory, and antimicrobial potentials and have a great diversity in Iran. In addition 54% of Iranian species of this genera are endemic plants and *Nepeta* species has a great place in traditional and ethno medicines (11).

In this study, cytoprotective and inhibition of NO effects of three endemic plants including *Scutellaria nepetifolia*, *Sc. multicaulis*, and *Nepeta laxiflora*, and another non-endemic plant named *Sc. tournefortii* have been investigated. None of the species could protect PC12 cells from oxidative stress, induced by hydrogen peroxide. However, *Sc. multicaulis* and *Sc. nepetifolia* effectively reduced NO levels in the stressed PC12 cells. In contrast, *Sc. tournefortii* increased the level of NO in oxidative stress condition. In previous studies, *Sc. multicaulis* was assessed for antioxidative, cytotoxic, and antiplasmodial effects. According to their results, it did not produce noticeable cytotoxic and antiplasmodial effects. Nevertheless, it had an average antioxidant action (18-20). There is no phytochemical study of *Sc. multicaulis* except for one study about essential oil composition that resulted in trans-Caryophyllene and Caryophyllene oxide as the main compounds (21). *Sc. tournefortii* was assessed for antioxidant effect and gave good results (22).

Unlike Iran's endemic *Scutellaria* spp., other species, especially *Scutellaria baicalensis* (*Sc. baicalensis*), were studied thoroughly for

their neuroprotective and antioxidant effects. *Sc. baicalensis* could protect PC12 cells from oxidative stress, induced by H₂O₂ (23). The flavonoids from *Sc. baicalensis* protected PC12 cells from amyloid β protein-induced neurotoxicity, too (24). As these studies on *Sc. baicalensis*, and baicalein as its main component, started about two decades ago, a vast number of *in-vitro* and *in-vivo* studies on its neurodegeneration and inflammation was established completely, and are available in literature in that field (25-29). We had expected cytoprotection from *Sc. nepetifolia* and *Sc. multicaulis*; however, they only could inhibit NO production effectively and did not protect PC12 cells from oxidative stress. Nevertheless, we must notice that the extracts were applied as pretreatment in this study and additional studies to examine cytoprotection in both treatment and pretreatment mode is recommended.

In contrast, *N. laxiflora* inhibit both cell death and NO production in stressed PC12 cells. Only one study was found in literature about *N. laxiflora* that examined antioxidant activity of essential oil which concluded that it produced a moderate effect (30). Still, there are many studies, which examine other *Nepeta* species' effects in various pathological conditions. Süntar *et al.* (11) thoroughly reviewed biological activities of *Nepeta* spp. and highlighted that *N. flavida* and *N. sintenisii* exerted moderate antioxidant activity. Other species like *N. cataria* and *N. clarkei* produced anti-inflammatory effects. Distinctively, based on this review, there is no specific study about *Nepeta* species cytoprotective and NO reducing effect in oxidative stressed cells. By noting, in the present study, that *N. laxiflora* is an Iranian endemic plant and protects PC12 cells from stress, this species is a good subject to supplementary neuroprotection studies.

References

- (1) Basudhar D, Somasundaram V, de Oliveira GA, Kesarwala A, Heinecke JL, Cheng RY, Glynn SA, Ambs S, Wink DA and Ridnour LA. Nitric oxide synthase-2-derived nitric oxide drives multiple pathways of breast cancer progression. *Antioxid. Redox Signal.* (2017) 26: 1044–58.

- (2) Boyd CS and Cadenas E. Nitric oxide and cell signaling pathways in mitochondrial-dependent apoptosis. *Biol. Chem.* (2002) 383: 411–23.
- (3) Reiniers MJ, van Golen RF, van Gulik TM and Heger M. Reactive oxygen and nitrogen species in steatotic hepatocytes: a molecular perspective on the pathophysiology of ischemia-reperfusion injury in the fatty liver. *Antioxid. Redox Signal.* (2014) 21: 1119–42.
- (4) Lee SR, Kim MS, Kim S, Hwang KW and Park SY. Constituents from *Scutellaria barbata* inhibiting nitric oxide production in LPS-stimulated microglial cells. *Chem. Biodivers.* (2017) 14: e1700231.
- (5) Zuo L, Koozechian MS and Chen LL. Characterization of reactive nitrogen species in allergic asthma. *Ann. Allergy Asthma Immunol.* (2014) 112: 18–22.
- (6) Perez-Hernandez J, Zaldivar-Machorro VJ, Villanueva-Porras D, Vega-Avila E and Chavarria A. A potential alternative against neurodegenerative diseases: phytodrugs. *Oxid. Med. Cell. Longev.* (2016) 2016: 8378613.
- (7) Braidly N, Behzad S, Habtemariam S, Ahmed T, Daglia M, Nabavi SM, Sobarzo-Sanchez E and Nabavi SF. Neuroprotective effects of citrus fruit-derived flavonoids, nobiletin and tangeretin in Alzheimer's and Parkinson's disease. *CNS Neurol. Disord. Drug Targets* (2017) 16: 387-97.
- (8) Orhan IE, Senol FS and Sener B. Recent approaches towards selected lamiaceae plants for their prospective use in neuroprotection. *Stud. Nat. Prod. Chem.* (2012) 38: 397-415.
- (9) Sadeghi Z, Akaberi M, Sobhkhizi A, Sahebkar A and Emami SA. Evaluation the ethno-pharmacological studies in Iran during 2004-2016: A systematic review. *J. Cell. Physiol.* (2018) 233: 914–23.
- (10) Shang X, He X, Li M, Zhang R, Fan P, Zhang Q and Jia Z. The genus *Scutellaria* an ethnopharmacological and phytochemical review. *J. Ethnopharmacol.* (2010) 128: 279–313.
- (11) Süntar I, Nabavi SM, Barreca D, Fischer N and Efferth T. Pharmacological and chemical features of *Nepeta* L. genus: Its importance as a therapeutic agent. *Phytother. Res.* (2018) 32: 185–98.
- (12) Clément MV, Ponton A and Pervaiz S. Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. *FEBS Lett.* (1998) 440: 13–8.
- (13) Sharma BV, Rowland NS, Clouse MM and Rice NA. An improved assay for measuring low levels of Nitric Oxide in cultured pulmonary myofibroblasts. *Adv. Biol. Chem.* (2014) 4: 214–21.
- (14) Behzad S, Ebrahim K, Mosaddegh M and Haeri A. *Primula auriculata* extracts exert cytotoxic and apoptotic effects against HT-29 human colon adenocarcinoma cells. *Iran. J. Pharm. Res.* (2016) 15: 311-22.
- (15) Lee DS, Ko W, Yoon CS, Kim DC, Yun J, Lee JK, Jun KY, Son I, Kim DW, Song BK, Choi S, Jang JH, Oh H, Kim S and Kim YC. KCHO-1, a novel antineuroinflammatory agent, inhibits lipopolysaccharide-induced neuroinflammatory responses through Nrf2-mediated heme oxygenase-1 expression in mouse BV2 microglia cells. *Evid. Based Complement. Alternat. Med.* (2014) 2014: 357154.
- (16) Alamdary SZ, Khodaghali F, Shaerzadeh F, Ansari N, Sonboli A and Tusi SK. *S. choleroleuca*, *S. mirzayanii* and *S. santolinifolia* protect PC12 cells from H₂O₂-induced apoptosis by blocking the intrinsic pathway. *Cytotechnology* (2012) 64: 403–19.
- (17) Tayarani-Najaran Z, Mousavi SH, Asili J and Emami SA. Growth-inhibitory effect of *Scutellaria lindbergii* in human cancer cell lines. *Food Chem. Toxicol.* (2010) 48: 599–604.
- (18) Esmaceli S, Naghibi F, Mosaddegh M, Sahranavard S, Ghafari S and Abdullah NR. Screening of antiplasmodial properties among some traditionally used Iranian plants. *J. Ethnopharmacol.* (2009) 121: 400–4.
- (19) Shamim S, Farzaneh N, Mahmoud M, Elaheh D, Hoong CY and Abdullah NR. Cytotoxic activity of some medicinal plants from Iran. *J. Ethnopharmacol.* (2009) 111: 657–66.
- (20) Soury E, Amin G, Dehmobed-Sharifabadi A, Nazifi A and Farsam H. Antioxidative activity of sixty plants from Iran. *Iran. J. Pharm. Res.* (2010) 3: 55–9.
- (21) Asadollahzadeh H and Rajaie N. Comparison of chemical composition of essential oils from aerial parts of *Scutellaria multicaulis* Boiss. using hydrodistillation, microwave-assisted hydrodistillation and headspace solid phase microextraction. *Trends Life Sci.* (2014) 3: 133–6.
- (22) Khalili M, Ebrahimzadeh MA and Safdari Y. Antihemolytic activity of thirty herbal extracts in mouse red blood cells. *Arh. Hig. Rada Toksikol.* (2014) 65: 399–406.
- (23) Shang YZ, Qin BW, Cheng JJ and Miao H. Prevention of oxidative injury by flavonoids from stems and leaves of *Scutellaria baicalensis* Georgi in PC12 cells. *Phytother. Res.* (2006) 20: 53–7.
- (24) Heo HJ, Kim DO, Choi SJ, Shin DH and Lee CY. Potent inhibitory effect of flavonoids in *Scutellaria baicalensis* on amyloid β protein-induced neurotoxicity. *J. Agric. Food Chem.* (2004) 52: 4128–32.
- (25) Heo H, Shin Y, Cho W, Choi Y, Kim H and Kwon YK. Memory improvement in ibotenic acid induced model rats by extracts of *Scutellaria baicalensis*. *J. Ethnopharmacol.* (2009) 122: 20–7.
- (26) Miao G, Zhao H, Guo K, Cheng J, Zhang S, Zhang X, Cai Z, Miao H and Shang Y. Mechanisms underlying attenuation of apoptosis of cortical neurons in the hypoxic brain by flavonoids from the stems and leaves of *Scutellaria baicalensis* Georgi. *Neural Regen Res.* (2014) 9: 1592–8.
- (27) Choi J, Conrad CC, Malakowsky CA, Talent JM, Yuan CS and Gracy RW. Flavones from *Scutellaria baicalensis* Georgi attenuate apoptosis and protein oxidation in neuronal cell lines. *Biochim. Biophys. Acta* (2002) 1571: 201–10.

- (28) Hwang YK, Jinhua M, Choi BR, Cui CA, Jeon WK, Kim H, Kim HY, Han SH and Han JS. Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion- induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. *J. Ethnopharmacol.* (2011) 137: 681–9.
- (29) Shang YZ, Qin BW, Cheng JJ and Miao H. Effect of *Scutellaria* flavonoids on KCN-induced damages in rat pheochromocytoma PC12 cells. *Indian J. Med. Res.* (2008) 127: 610–5.
- (30) Safaei-Ghomi J, Nahavandi S and Batooli H. Studies on the antioxidant activity of the volatile oil and methanol extracts of *Nepeta laxiflora* Benth. and *Nepeta Sessilifolia* Bunge. from Iran. *J. Food Biochem.* (2011) 35: 1486–92.

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