ORIGINAL ARTICLE

Carthamus, Salvia and Stachys species protect neuronal cells against oxidative stress-induced apoptosis

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Abstract

Context: Finding effective therapies for neurodegenerative diseases is of utmost importance for the aging population. Plants growing in Iran are rich sources of antioxidants and active phytochemicals.

Objective: The protective capacity of plants, with a special focus on those with reported antioxidant or neuroprotective potential or nervous system-related applications in folk medicine, was tested against oxidative stress-induced apoptosis.

Materials and methods: Aerial parts of 20 plants including *Carthamus*, *Salvia*, and *Stachys* species were extracted with 80% methanol and dichloromethane and preincubated with neuronal PC12 cells for 3 h. Oxidative stress and apoptosis were induced by hydrogen peroxide (75 μM, 1 h exposure). Cell viability and intracellular reactive oxygen species (ROS) were measured by MTT and 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) assays, respectively, while apoptosis was determined by annexin V-FITC/propidium iodide staining by a flow cytometer.

Results: Eighty percent methanol extracts of *Carthamus oxyacantha* Bieb. (Asteraceae), *Salvia santolinifolia* Boiss. (Lamiaceae), and *Salvia sclarea* L. (Lamiaceae) at the concentration of 100 μg/ml showed significant neuroprotection in the MTT assay by 38.7, 34.7, and 39.5%, respectively, and inhibited intracellular ROS by 48.6, 61.9, and 61.4%, respectively. The first two extracts also significantly inhibited apoptosis. Dichloromethane extracts of *C. oxyacantha* and *Stachys pilifera* Benth. (Lamiaceae) at the concentration of 25 μg/ml showed neuroprotection by 27.5 and 26.5%, respectively, and inhibited ROS by 44.5 and 39.4%, respectively.

Conclusion: The above-mentioned plants seem to have important biological activities and their further study may lead to the discovery of new natural therapeutics useful against disorders such as Alzheimer and Parkinson diseases.

Introduction

The incidence of neurodegenerative disorders, particularly Alzheimer and Parkinson diseases, rapidly increases with age and, therefore, these pathologies represent important health challenges for all aging populations in the world (Ritchie & Lovestone, 2002). Currently, there is no disease-modifying treatment strategy for most neurodegenerative disorders. Therefore, the study of compounds that target basic mechanisms of disease is of utmost importance for the discovery of novel therapeutic agents against neurodegeneration (Lang, 2010).

Alzheimer and Parkinson diseases both belong to the category of protein-misfolding disorders; amyloid-β oligomers and fibrils as well as hyperphosphorylated Tau protein aggregates are involved in the pathogenesis of Alzheimer's (Querfurth & LaFerla, 2010), while α-synuclein is one of the crucial pathological elements of Parkinson disease (Winner et al., 2011). However, in both of these conditions, as well as in other neurodegenerative diseases, oxidative stress is believed to be among one of the basic mechanisms that contributes to the process of neurodegeneration (Clark et al., 2010).

Oxidative stress, defined as the failure of antioxidant defense mechanisms in mending the harmful effects of reactive oxygen species (ROS) (Sies, 1997), is an important process that can lead to neuronal cell damage in neurodegenerative diseases (Melo et al., 2011). In this regard, many studies have focused on the use of antioxidants for the management of neurodegenerative diseases (Firuzi et al., 2011).

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Plants are rich sources of bioactive compounds. Folk medicine of different countries contains a wealth of helpful information regarding the usefulness of plants for certain medical conditions (Howes & Houghton, 2003). Several studies have confirmed that different plants, especially those used in traditional medicine, possess antioxidant properties (Matkowski & Piotrowska, 2006) and protective effects against various toxins in cell models including cell models of neurodegenerative diseases (Iuvone et al., 2006). Furthermore, plant-derived antioxidant/neuroprotective compounds may satisfy the requirements of pleiotropic or multi-target drugs that seem to be much needed in the field of neurodegenerative diseases (Frautschy & Cole, 2010; Schubert & Maher, 2012).

Plants used in traditional Iranian medicine have also been studied for their antioxidant (Firuzi et al., 2010a,b; Yazdanparast et al., 2008) and neuroprotective properties (Mousavi et al., 2010). Particularly, the protective effects of plants from the genus *Salvia* against oxidative stress induced damage in PC12 cells have been demonstrated by several investigators (Alamdary et al., 2012; Asadi et al., 2010, 2011).

In this study, several plants that grow wild in different areas of Iran were collected. They were selected either from the genera with high antioxidant potential or from plants used in traditional medicine for neurological disorders and related conditions. Selected plants were extracted with two different methods in order to include compounds with different lipophilicities. The extracts were then tested in a cell model of neurodegenerative diseases and plant extracts that could inhibit oxidative stress-induced cytotoxicity were further studied for their capacity in inhibition of intracellular ROS formation and apoptosis.

Materials and methods

Reagents

Fetal bovine serum (FBS), penicillin/streptomycin, RPMI 1640, sterile phosphate-buffered saline (PBS), and trypsin EDTA 0.05% were purchased from Biosera (Ringmer, UK), while 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), hydrogen peroxide, and *N*-acetylcysteine were obtained from Sigma-Aldrich (St Louis, MO). Horse serum was acquired from Invitrogen (San Diego, CA), while dimethyl sulfoxide and methanol were obtained from Merck (Darmstadt, Germany). Annexin-V-FITC/propidium iodide (PI) apoptosis detection kit was obtained from BD Pharmingen, Franklin Lakes, NJ. Deionised water (conductivity $<0.1\,\mu\mathrm{S\,cm^{-1}}$) was used in all the experiments.

Plants collection and extraction

Seeds of *Bunium persicum* Boiss. (Apiaceae), Persian name: Zireh kuhi, flowers of *Echium amoenum* Fisch. and C.A. Mey (Boraginaceae), Persian name: Gol-e-Gavzaban, and seeds of *Heracleum persicum* L. (Apiaceae), Persian name: Golpar were purchased from local herbal stores in the city of Shiraz, while the other plants were collected from different regions of Iran as described in Table 1 and identified by a botanist, Mojtaba Asadollahi.

Aerial parts of collected plants were air-dried in the shade and then powdered. The powder was separately extracted by methanol:H_2O (80:20) and dichloromethane as follows; the powdered plant (1 g) was soaked in 20 ml of solvent overnight. The solvent was removed by rotary evaporation and the extracts were dried in a freeze dryer. This extract was dissolved in DMSO at the concentration of 40 mg/ml, aliquoted and kept at $-80\,^{\circ}$C until use.

Cell culture

PC12 cells (rat pheochromocytoma) were a generous gift from Professor Lloyd A. Greene (Department of Pathology and Cell Biology, Columbia University, New York, NY). Cells were maintained in RPMI 1640 supplemented with 10% horse serum, 5% FBS, 100 U/ml penicillin G, and 100 μg/ml streptomycin. They were cultured on plates coated with collagen obtained from rat tail and were grown at 37 °C in

Table 1. Locations of collection and herbarium numbers of the plants that were gathered from different regions of Iran.

Plant name	Family	Location	Herbarium number	Date
Achillea wilhelmsii C. Koch	Asteraceae	Fars province: Shiraz, Bajgah, Bamoo mountain.	PC-88-51	June 2008
Carthamus oxyacantha Bieb.	Asteraceae	Fars province: Shiraz, Ghalat.	PC-88-61	June 2008
Salvia aethiopis L.	Lamiaceae	East Azarbaijan province: Arasbaran Forest.	PC-87-91	August 2008
Salvia atropatana Bunge	Lamiaceae	Fars province: Sepidan, Cheleghah.	PC-88-19	July 2008
Salvia eremophila Boiss.	Lamiaceae	Fars province: Darab.	PC-87-92	June 2008
Salvia nemorosa L.	Lamiaceae	Mazandaran province: Marzanabad, Chalus.	PC-88-20	July 2008
Salvia santolinifolia Boiss.	Lamiaceae	Fars province: Darab.	PC-87-98	June 2008
Salvia sclarea L.	Lamiaceae	Fars province: Sepidan, Komehr.	PC-87-99	July 2008
Salvia xanthocheila Boiss. ex Benth.	Lamiaceae	Mazandaran province: Kandovan-Chalus road.	PC-87-101	July 2008
Sambucus ebulus L.	Adoxaceae	Gilan province: Sowme'eh Sara.	PC-88-58	July 2008
Stachy acerosa Boiss.	Lamiaceae	Fars province: Dashte Arjan.	PC-88-14	June 2008
Stachys byzantina C. Koch	Lamiaceae	Gilan province: Asalem-Khalkhal road.	PC-88-16	July 2008
Stachys obtusicrena Boiss.	Lamiaceae	Kohgiluyeh province: Dena mountain, GardanehBijan.	PC-88-15	June 2008
Stachys pilifera Benth.	Lamiaceae	Fars province: Sepidan to Margoon waterfall.	PC-88-17	June 2008
Stachys spectabilis Choisy ex DC.	Lamiaceae	Fars province: Sepidan, Chelegah.	PC-88-10	June 2008
Zataria multiflora Boiss.	Lamiaceae	Fars province: Fasa, Mianjangal.	PC-90-11	June 2008
Ziziphora clinopodioides Lam.	Lamiaceae	Kohgiluyeh province: Dena mountain.	PC-88-65	July 2008

In addition to the 17 plants listed in this table, another three plants including *Bunium persicum* Boiss. (Apiaceae), *Echium amoenum* Fisch. and C.A. Mey (Boraginaceae) and *Heracleum persicum* L. (Apiaceae) were purchased from local herbal stores.

humidified air containing 5% CO_2. Two-thirds of the growing medium was changed every 2–3 d and the cells were subcultured roughly once a week.

Measurement of cytotoxicity by the MTT assay

Cell viability following exposure to hydrogen peroxide was determined by the MTT reduction assay (Garrido et al., 2012). MTT is absorbed by viable cells and is converted to formazan by the enzyme succinate dehydrogenase in the mitochondria. The amount of produced formazan thus correlates with the number of living cells. Hydrogen peroxide (8.8 M solution) was first diluted in PBS to prepare a 100 mM solution on the day of the experiment. This was further diluted in a growth medium to prepare the final working solution. PC12 cells were seeded in collagen-coated 96-well microplates at a density of 5×10^5 cells/ml (100 μl per well). Blank wells contained only the growth medium for background correction. After 48 h of incubation that ensured proper cell attachment, 20 μl of the growth medium supplemented with different concentrations of plant extracts (10–100 μg/ml final concentration in the well) were added in triplicate and incubated for 3 h. Maximum concentration of DMSO in the wells was kept below 0.2%. The cells were challenged with H_2O_2 at the final concentration of 75 μM for 1 h and the medium was replaced with fresh one and cells were incubated overnight. In the end, the medium was replaced with 20 μl of MTT 0.5 mg/ml dissolved in RPMI. After 1.5 h at 37 °C, formazan crystals were solubilized in 200 μl DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680, Bio-Rad Ultramark Microplate Imaging System, Sun Valley, CA). Each experiment was repeated 4–6 times. The percent inhibition of cell damage was calculated with a reference to the absorbance of control wells not challenged with hydrogen peroxide (assumed as 100% protection) and the absorbance of wells treated with hydrogen peroxide in the absence of any plant extract (assumed as 0% protection). *N*-acetylcysteine was also tested as a reference antioxidant compound.

Determination of intracellular ROS by DCFH-DA

The fluorescent probe DCFH-DA was used to monitor intracellular accumulation of ROS (Bass et al., 1983). PC12 cells were seeded in collagen-coated 24-well plates at a density of 4×10^5 cells/ml (500 μl per well) and incubated for 72 h. Different concentrations of plant extracts were then added in a duplicate for 3 h. DCFH-DA (10 μM) was added and after 30 min of incubation at 37 °C in the dark, the media were replaced with 300 μl of PBS. Fluorescence emission was measured (e_0) at the excitation and the emission wavelength of 485 nm and 520 nm, respectively, by a multi-mode microplate reader (BMG Labtech, Cary, NC). Afterwards, the cells were treated with H_2O_2 100 μM and the fluorescence intensity was measured again after 60 min (e_{60}). The difference between e_0 and e_{60} was calculated (ΔF). Inhibition of ROS in the presence of various concentrations of plant extracts was calculated by the following formula:

$$\%\text{ROS inhibition} = 100 \times \frac{\left(\begin{array}{c} \Delta F \text{ oxidized control} \\ -\Delta F \text{ sample} \end{array} \right)}{\left(\begin{array}{c} \Delta F \text{ oxidized control} \\ -\Delta F \text{ non-oxidized control} \end{array} \right)}$$

Detection of apoptotic cells with flow cytometry

Apoptotic cells were detected and quantified by an Annexin V-FITC/PI apoptosis detection kit (Sigma-Aldrich, St. Louis, MO). The cells were seeded in 6-well plates at a density of 5×10^5 cells/ml, and after 48 h, plant extracts (100 μg/ml) were added in duplicate and incubated for 3 h before exposure to H_2O_2 for 1 h. Afterwards, the cells were trypsinized and resuspended in 1000 μl binding buffer at a density of 1×10^6 cells/ml. The cells were then stained according to the manufacturer's instruction and analyzed by a BD FACSCalibur flow cytometer (Yu et al., 2008). The sum of percentages of lower right (LR) and upper right (UR) quadrants was considered as the percentage of apoptotic cells.

Statistical analysis

Comparisons between different groups were performed by a one-way analysis of variance (ANOVA) by SPSS software (version 11.5.0 for Windows; SPSS Inc., Chicago, IL). Differences with *p* values of less than 0.05 were considered statistically significant.

Results

Protection of PC12 cells against H_2O_2-induced cytotoxicity

The capacity of plant extracts in protection of neuronal PC12 cells against hydrogen peroxide-induced cytotoxicity was measured by MTT cell viability assay. H_2O_2 reduced cell viability to 40.8% compared with the control. Percent inhibition of cytotoxicity was calculated for each concentration of the extracts. *N*-Acetylcysteine was used as a positive control (Figure 1).

Eighty percent methanol extracts of *Carthamus oxyacantha* Bieb. (Asteraceae), *Salvia santolinifolia* Boiss. (Lamiaceae), and *Salvia sclarea* L. (Lamiaceae) and also dichloromethane extracts of *C. oxyacantha* and *St. pilifera* Benth. (Lamiaceae) showed significant inhibition of cytotoxicity induced by hydrogen peroxide at different concentrations.

Attenuation of H_2O_2-induced formation of intracellular ROS

Intracellular ROS were measured by DCFH-DA. This probe is hydrolyzed inside the cell and gains fluorescence after being oxidized by ROS. Plants that demonstrated protective effects in the MTT assay were also examined by this method. Fluorescence emission was increased from 4135.3 RFU in non-oxidized cells to 17 222.9 RFU in cells exposed to 100 μM H_2O_2.

Figure 1. Inhibition of H_2O_2-induced cyto-toxicity in PC12 cells by plant extracts. PC12 cells seeded in collagen-coated 96-well microplates were pre-incubated with different concentrations of 80% methanol and dichloromethane (DCM) plant extracts for 3 h and then challenged with H_2O_2 75 μM for 1 h. MTT assay was performed to measure cell viability after 24 h. Each experiment was repeated 4–6 times. Only active plant extracts are depicted in the figure. Concentrations of plant extracts and NAC (positive control) are expressed as μg/ml and μM, respectively. Data are expressed as mean ± S.E.M. *Significantly different from control wells treated with hydrogen peroxide ($p < 0.05$). *C.O.*: *Carthamus oxyacantha*; *S. Sa*: *Salvia santolinifolia*; *S. Sc*: *Salvia sclarea*; *St. P.*: *Stachys pilifera*; NAC: *N*-acetylcysteine.
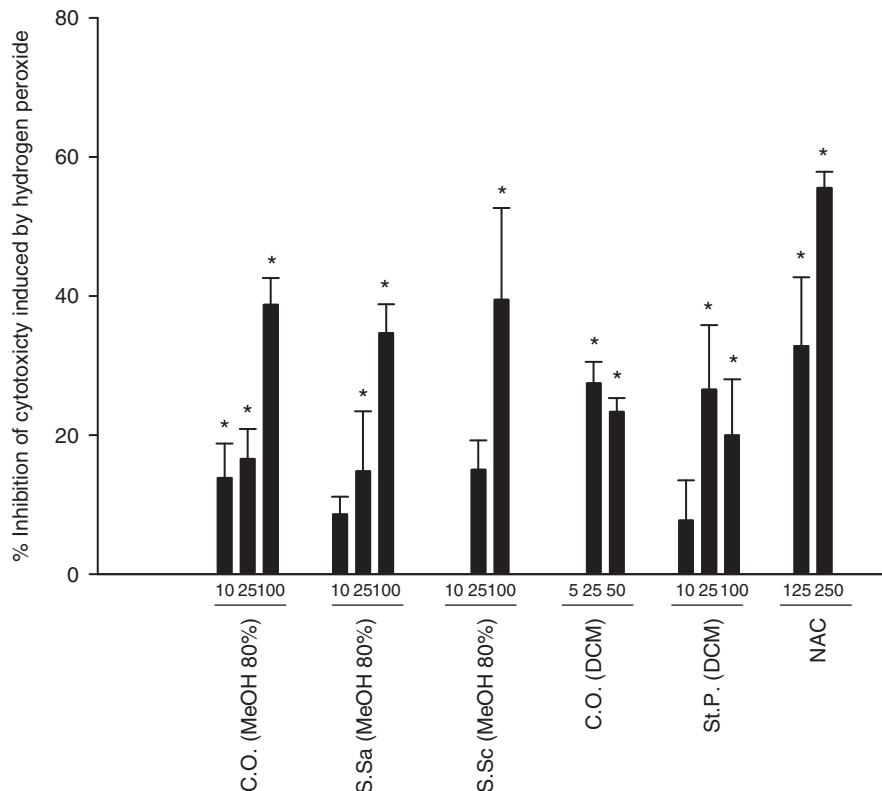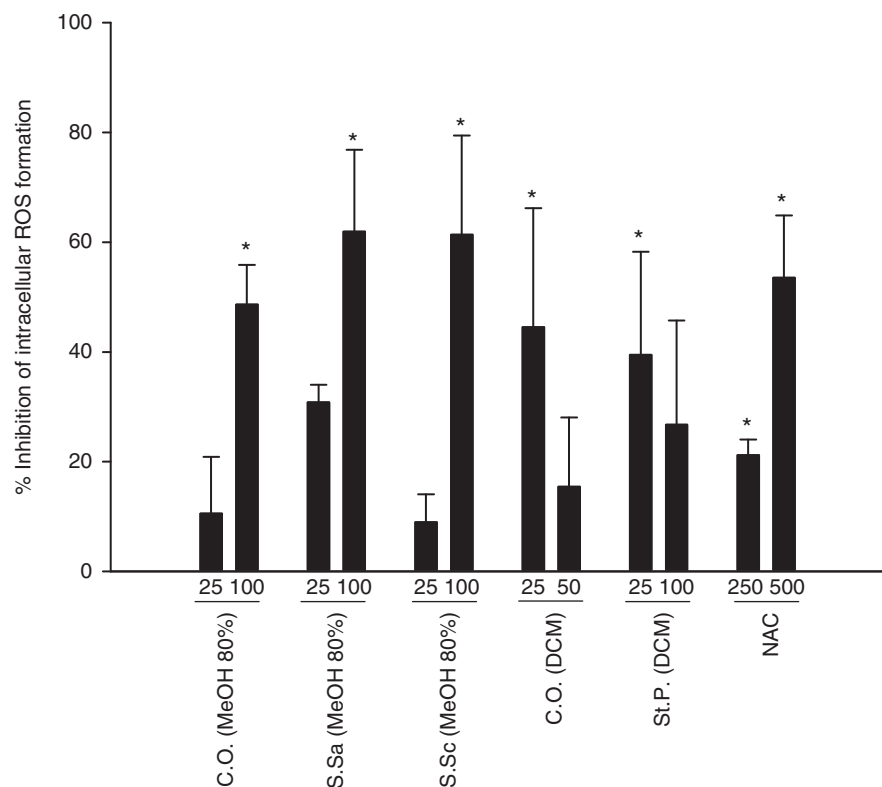

Figure 2. Inhibition of H_2O_2-induced intra-cellular reactive oxygen species (ROS) formation in PC12 cells by plant extracts. PC12 cells seeded in collagen-coated 24-well plates were incubated with different concentrations of 80% methanol and dichloromethane (DCM) plant extracts for 3 h and then 10 μM DCFH-DA for 30 min. H_2O_2 100 μM was added and fluorescence emission was measured before and after H_2O_2 and %ROS inhibition was calculated as described in the text. Each experiment was repeated 3–6 times. Concentrations of plant extracts and NAC (positive control) are expressed as μg/ml and μM, respectively. Data are expressed as mean ± S.E.M. *Significantly different from control wells treated with hydrogen peroxide ($p < 0.05$). *C.O.*: *Carthamus oxyacantha*; *S. Sa*: *Salvia santolinifolia*; *S. Sc*: *Salvia sclarea*; *St. P.*: *Stachys pilifera*. NAC: N-acetylcysteine.

Eighty percent methanol extracts of *C. oxyacantha*, *S. santolinifolia*, and *S. sclarea* at the concentration of 100 μM significantly inhibited intracellular ROS production induced by hydrogen peroxide (Figure 2). Dichloromethane extracts of *C. oxyacantha* and *St. pilifera* were also able to show a modest inhibition of intracellular ROS formation at 25 μM; however, similar to the MTT assay,

the use of a higher concentration did not increase the effect (Figure 2).

Inhibition of H_2O_2-induced apoptosis

Flow cytometric detection of apoptosis caused by H_2O_2 in PC12 cells was performed by Annexin V and PI double
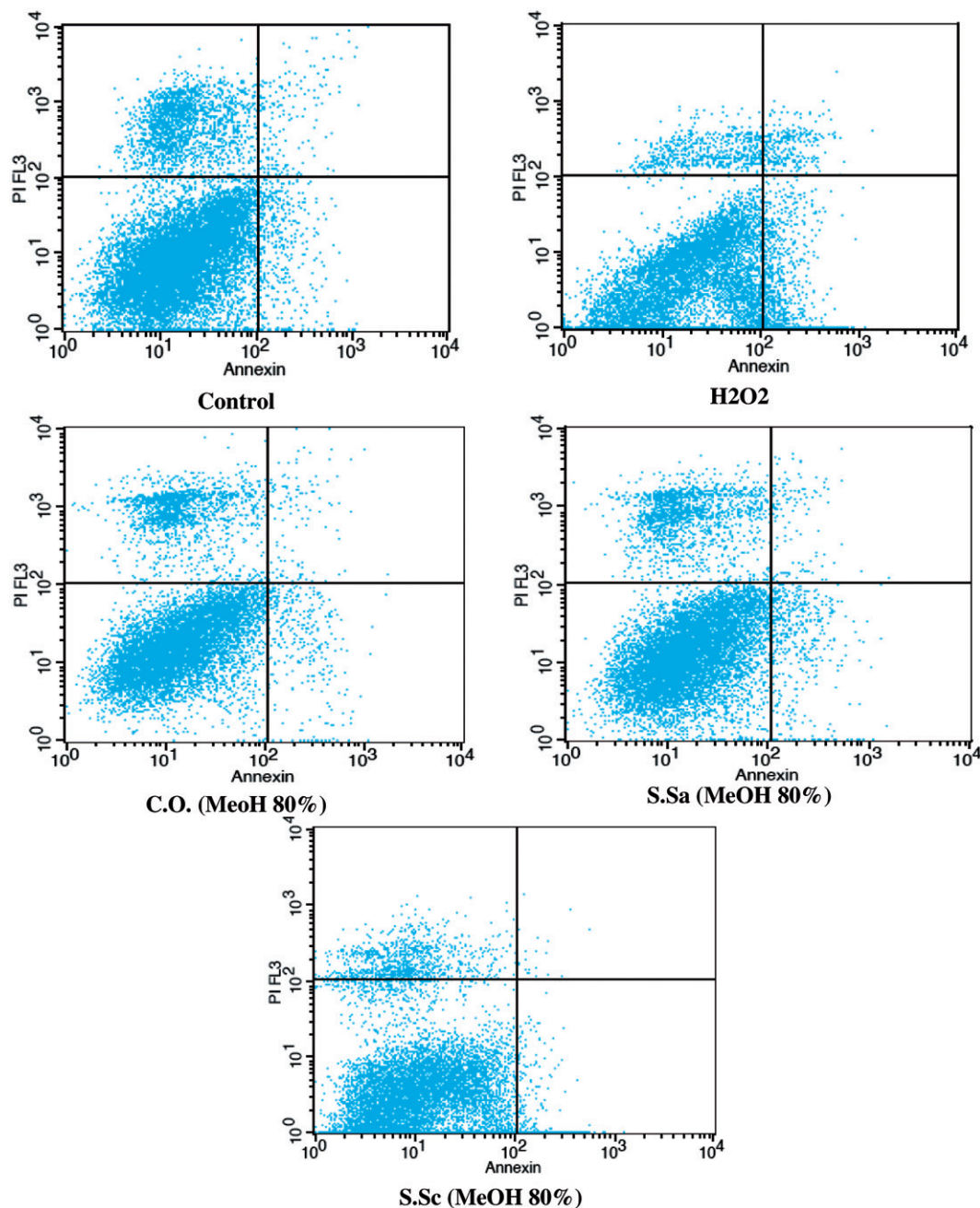
Figure 3. Inhibition of H_2O_2-inducd apoptosis in PC12 cells by plant extracts. Apoptosis was measured by an Annexin V/propidium iodide (PI) kit (Sigma-Aldrich, St. Louis, MO). The cells were seeded in 6-well plates and incubated with plant extracts (100 µg/ml) for 3 h and then exposed to H_2O_2 for 1 h before being processed for apoptosis detection by a flow cytometer. *C.O.*: *Carthamus oxyacantha*; *S. Sa*: *Salvia santolinifolia*; *S. Sc*: *Salvia sclarea*.

staining. Eighty percent methanol extracts of *C. oxyacantha*, *S. santolinifolia*, and *S. sclarea* that could significantly inhibit cell damage and oxidative stress in MTT and DCFH-DA assays were tested by this method. *Carthamus oxyacantha* and *S. santolinifolia* significantly decreased the percentage of apoptotic cells (Figures 3 and 4).

Discussion

Twenty plants growing in Iran were analyzed for their neuroprotective capacity in a cell model of oxidative stress-induced apoptosis. Eighty percent methanol and dichloro-methane extracts were prepared from all plants to encompass

compounds with a range of different polarities. Eighty percent methanol extracts of *C. oxyacantha*, *S. santolinifolia,* and *S. sclarea* and also dichloromethane extracts of *C. oxyacantha* and *St. pilifera* showed significant inhibition of cytotoxicity induced by hydrogen peroxide in PC12 cells and the same extracts also significantly inhibited intracellular ROS production. Eighty percent methanol extracts of *C. oxyacantha* and *S. santolinifolia* significantly inhibited hydrogen peroxide-induced apoptosis. Other tested plants showed no effect in these assays. Plants that were selected for this study were either those with high antioxidant activities identified in our previous reports (Firuzi et al., 2010b; Jassbi et al., 2014) or medicinal plants such as *S. sclarea* (Asadi et al., 2010;
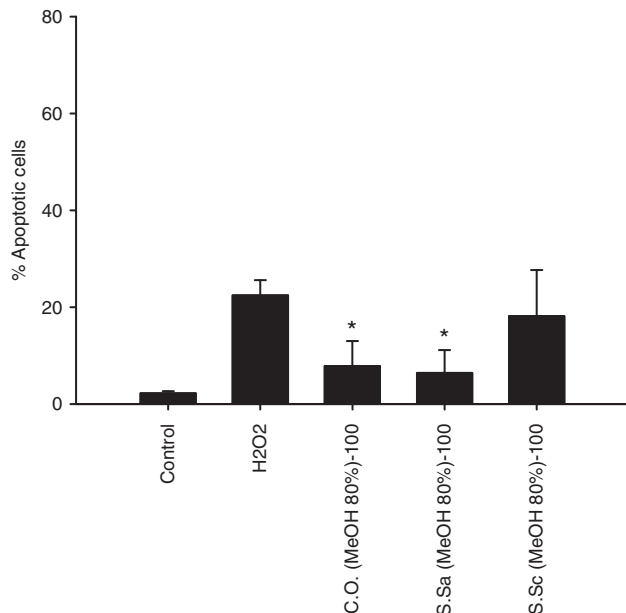
Figure 4. Inhibition of H_2O_2-inducd apoptosis in PC12 cells by plant extracts. Apoptosis was evaluated by an Annexin V/propidium iodide (PI) kit using a flow cytometer. The sum of lower right and upper right quadrants (as in Figure 3) was considered as the percentage of apoptotic cells. Data are expressed as mean ± S.E.M. *Significantly different from control wells treated with hydrogen peroxide ($p < 0.05$). *C.O.*: *Carthamus oxyacantha*; *S. Sa*: *Salvia santolinifolia*; *S. Sc*: *Salvia sclarea*.

Tepe et al., 2006) and *St. pilifera* (Farjam et al., 2011), which also possess antioxidant activity. Other investigators have previously suggested that screening of plants that are used in the traditional medicine has a higher chance of finding bioactive compounds (Gyllenhaal et al., 2012). Therefore, we also included medicinal plants that are used for neurological disorders in the folk medicine such as *E. amoenum* (Amin, 2005) as well as those used for other medical purposes including *Achillea wilhelmsii* C. Koch (Asteraceae) (Hasani-Ranjbar et al., 2010), *B. persicum* (Amin, 2005), *C. oxyacantha* (Ahmad et al., 2009), *H. persicum* (Amin, 2005), *Sambucus ebulus* L. (Adoxaceae) (Quave et al., 2011), *St. Byzantina* C. Koch (Lamiaceae) (Duarte et al., 2005), *Zataria multiflora* Boiss. (Lamiaceae) (Amin, 2005), and *Ziziphora clinopodioides* Lam. (Lamiaceae) (Amin, 2005), which are more likely to have reasonable biological activities.

One possible explanation as to why most plants did not show a neuroprotective effect in our assays could reside in the fact that plant extracts contain a plethora of various compounds and some of them may indeed induce apoptosis in the cells (González-Sarrías et al., 2012; Singh et al., 2012), an effect that may have masked the neuroprotective capacity in our cell-based assay. In contrast, the pro-oxidant effects of some polyphenolic compounds that are abundantly present in plant extracts could be another explanation for this observation (Macakova et al., 2012; Prochazkova et al., 2011). *Carthamus oxyacantha*, that is known as wild safflower in the traditional medicine, has antioxidant and anti-hyperlipidemic properties (Ahmad et al., 2009), but its neuroprotective effect has not been previously reported. Both extracts of this plant (80% methanol and dichloromethane) exhibited a neuroprotective effect in the MTT assay and inhibited

intracellular ROS accumulation. Further, 80% methanol extract of *C. oxyacantha* significantly inhibited apoptosis induced by hydrogen peroxide.

In a recent report, an unusual spiro sesquiterpene and four other related compounds were isolated from the methanol extract of *C. oxyacantha* and their structures were determined by different spectroscopic methods (Johansen et al., 2011). In addition to these novel compounds, some known derivatives of caffeic acid, vanillic acid, and different flavonoids have also been detected in this plant by LC-MS (Johansen et al., 2011), which may be the reason for its neuroprotective activity in our study.

Our results and other investigators' findings introduce *C. oxyacantha* as a good candidate for further studies in a bioassay guided purification to find its active natural products.

The other plant in our study with a considerable neuroprotective profile was *S. santolinifolia*, which its methanol 80% extract was active in all three applied methods. We have previously reported high antioxidant properties (Firuzi et al., 2010b, 2013) and other investigators have shown inhibition of apoptosis in PC12 cells for this plant (Asadi et al., 2011; Alamdary et al., 2012). In a previous investigation, HPLC analysis identified compounds such as catechins and rosmarinic acid in the methanolic extract of this plant (Asadi et al., 2011), which may well be responsible for the observed neuroprotective effect of this plant.

Salvia sclarea, a medicinal plant also known as clary sage, was another plant with reasonable activity in our experiments. Our findings confirm previous reports about antioxidant and neuroprotective capacity of this plant (Asadi et al., 2010; Firuzi et al., 2013).

Previous studies have shown that *S. sclarea* acetone extract contains diterpenes, sesquiterpenes, and several flavonoids (Ulubelen et al., 1994). Among these components, flavonoids may be responsible for the antioxidant and neuroprotective effect of *S. sclarea*. Sclareol, a diterpene compound, which is also found in this plant extract (Ulubelen et al., 1994), possesses several biological activities (Noori et al., 2013) and it can increase the activity of antioxidant enzymes (Huang et al., 2012).

Stachys pilifera is a medicinal plant that is endemic to Iran and has been investigated for its antioxidant properties (Viswanathan et al., 2009). Butanol extract of this plant, which contains more lipophilic agents, has antitumor properties, probably through induction of apoptosis (Viswanathan et al., 2009). We observed inhibition of cytotoxicity and intracellular ROS formation at the lower concentration of 25 μM for this extract; however, these effects were not increased by the use of a higher concentration of 100 μM (Figures 1 and 2). This finding may be due to the presence of cytotoxic and pro-oxidant compounds that could induce apoptosis at high concentrations of the plant extract (Macakova et al., 2012; Prochazkova et al., 2011; Singh et al., 2012).

Essential oil composition of *St. pilifera* has been previously elucidated (Sefidkon & Shaabani, 2004); however, non-volatile components of this plant's extracts have not been studied yet.

Another finding of our study was that, in general, methanol–water extracts of plants had a better protective

effect compared with dichloromethane extracts. This may indicate that more polar compounds present in these plants are probably more effective in conditions of our assays.

The blood–brain barrier (BBB) crossing is an important parameter to be considered in drug discovery in the field of neurodegenerative diseases. There are multiple factors that determine the ability of a compound in crossing the BBB, among which molecular weight (<400 KD), lipid solubility, and the number of hydrogen bond donors and acceptors are the most important parameters (Pardridge, 2012). Compounds present in 80% methanol extracts are in general less lipophilic compared with those present in dichloromethane extracts and, therefore, less favorable from this aspect. However, 80% methanol extract also contains a vast range of various compounds with different lipophilicities, among which, several agents with favorable lipophilicity profiles could also be present.

Clarification on the nature of compounds responsible for the neuroprotective ability of these plants needs further studies; however, previous reports on the other members of the genus *Carthamus*, *Salvia*, and *Stachys* may provide some clues in this aspect. It has been shown that compounds such as Safflor yellow and hydroxysafflor yellow in *Carthamus tinctorius* L. (Wang et al., 2009; Yang et al., 2010) and salvionic acid B (Tian et al., 2008), tanshinone IIA (Liu et al., 2010), and cryptotanshinone (Mahesh et al., 2012) in *Salvia miltiorrhiza* and salvigenin (Rafatian et al., 2012) in various *Salvia* species can bring about neuroprotection. It is likely that some of these natural compounds are present in the active plants of the present study.

Conclusion

Screening of various plants growing in Iran showed that some of them have significant potential for inhibition of oxidative stress-induced neuronal cell damage in a well-established cell model of neurodegeneration. It seems that these plants are rich sources of biologically active natural products and their further investigation may potentially lead to the discovery of new plant derived compounds to be used for the management of neurodegenerative diseases.

Acknowledgements

Declaration of interest

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