Food Chemistry 115 (2009) 37–42

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Protective effects of four Iranian medicinal plants against free radical-mediated protein oxidation

Seifollah Bahramikia, Amin Ardestani, Razieh Yazdanparast *

Institute of Biochemistry and Biophysics, P.O. Box 13145-1384, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 12 May 2008
Received in revised form 6 October 2008
Accepted 17 November 2008

Keywords:
Protein oxidation
Teucrium polium
Antioxidant activities
Fe^{+2}/ascorbate
Reactive oxygen species

ABSTRACT

The role of oxidative protein damages in the pathophysiology of human diseases is currently a topic of considerable interest as oxidised proteins has been implicated in a wide spectrum of clinical disorders. In this study, the antioxidant activities of four Iranian medicinal plants, namely *Teucrium polium*, *Cyperus rotundus*, *Anethum graveolens* and *Nasturtium officinale* against metal–catalysed protein oxidation were evaluated by pro-oxidant model (Fe^{2+}/ascorbate) in rat liver homogenates. The addition of Fe^{2+}/ascorbate to the liver homogenate significantly increased the extent of protein oxidation, such as protein carbonyl (PCO) formation and loss of protein-bound sulphydryl (P-SH) groups. Furthermore, the rates of reactive oxygen species (ROS) formation and lipid peroxidation (LPO) were also increased. The plant extracts showed inhibitory effects against PCO formation, P-SH oxidation, ROS formation and LPO to varying degrees. Based on this study, the order of antioxidant activity against protein oxidation was found to be: *T. polium* > *C. rotundus* > *A. graveolens* > *N. officinale*. The protective effects of each plant extract could be due to its polyphenolic content. In that respect, the *T. polium* extract with highest polyphenolic content has more antioxidant activity against protein oxidation.

1. Introduction

Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defence system, which results in functional tissue damage. Reactive oxygen species (ROS) including free radicals in the forms of superoxide anion (O_2^-), hydroxyl radicals ($^{\cdot}OH$), singlet oxygen ($^1O_2$) and non-free radical species, such as hydrogen peroxide (H_2O_2), are various forms of activated oxygen, resulting from oxidative biological reactions or exogenous factors. They are very transient species, due to their high chemical reactivity, and cause destructive and irreversible damages to cellular components including proteins, lipids and DNA (Halliwell & Gutteridge, 1999). Oxidative damage, due to unregulated production of ROS, has been implicated in many clinical disorders, such as atherosclerosis, diabetes, cancer and neurodegenerative diseases (Aruoma, 1998). Mechanisms responsible for the ROS-mediated injuries to cells and tissues mainly include lipid peroxidation, oxidative DNA damage and protein oxidation. As a result, lipid and DNA oxidation have been the focus of investigations for many years. However due to their relatively high abundance, it is now recognised that proteins and/or proteins and lipids are the main targets for oxidants (Kayali, Cakatay, & Tekeli, 2007). The role of oxidative protein damage in the pathophysiology of human diseases is currently a topic of considerable interest, as oxidised proteins has been implicated in a wide spectrum of clinical conditions such as, diabetes, atherosclerosis, Alzheimer's disease, chronic lung disease, chronic renal failure, acute pancreatitis and sepsis (Dalle-Donne, Giustarini, Colombo, Rossi, & Milzani, 2003; Telci, Cakatay, Salman, Satman, & Sivas, 2000). Radical-mediated damage to proteins may be initiated by electron leakage, metal–ion dependent reactions and autoxidation of lipids and sugars (Dean, Fu, Stocker, & Davies, 1997). Accumulation of modified proteins disrupts cellular function, either by loss of catalytic and structural integrity or by interruption of regulatory pathways. It is well established that exposure of proteins to ROS can alter the physical and chemical structure of the target causing consequent oxidation of side chain groups, backbone fragmentation, cross-linking, unfolding and formation of new reactive groups (Dean et al., 1997; Kayali et al., 2007; Stadtman & Levin, 2000). Major molecular mechanisms leading to structural changes in proteins are metal-catalysed protein oxidation, characterised by protein carbonyl (PCO) formation and loss of protein thiol (P-SH) groups (Stadtman & Levin, 2000; Telci et al., 2000).

Abbreviations: DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DNPH, dinitrophenylhydrazine; DTNB, 5,5′-dithiobisnitro benzoic acid; FCR, Folin–Ciocalteu's reagent; P-SH, protein-bound sulphydryl; PCO, protein carbonyl; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

* Corresponding author. Tel.: +98 21 66956976; fax: +98 21 66404680.
E-mail address: yazdan@ibb.ut.ac.ir (R. Yazdanparast).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.11.054

Table 1
Name (family), locations, collection periods, voucher herbarium specimens and traditional uses of four Iranian medicinal plants.

Name (family)	Localities and collection periods	Voucher herbarium specimen	Traditional uses
T. polium L. (Lamiaceae)	Fars, Iran, spring 2006	No. 570, The Herbarium of the School of Pharmacy, Shaeed Beheshti University, Tehran, Iran	Anti-diabetic, anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive and anti-hyperlipidaemic
C. rotundus L. (Cyperaceae)	Tehran, Iran, spring 2005	No. 15299, The Central Herbarium of University of Tehran, Tehran, Iran	Anti-diabetic, anti-diarrhoea, anti-pyretic, anti-inflammatory, anti-malaria and also for treatment of stomach and bowel disorders
A. graveolens L. (Appiaceae)	Lorestan, Iran, spring 2006	No. 11018, The Central Herbarium of University of Tehran, Tehran, Iran	Anti-hyperlipidaemic, gastrointestinal ailments such as flatulence, indigestion, stomach ache and colic to tract intestinal gas
N. officinale R. Br. (Brassicaceae)	Lorestan, Iran, spring 2006	No. 39055, The Central Herbarium of University of Tehran, Tehran, Iran	Depurative, diuretic, expectorant, hypoglycaemic and odontalgic

In order to cope with the excess of free radicals produced upon oxidative stress, humans have developed sophisticated mechanisms in order to maintain redox homeostasis. These mechanisms include enzymatic and non-enzymatic antioxidant systems to scavenge or detoxify ROS, block their production or sequester transition metals which are the source of free radicals (Chaudiere & Ferrari-Iliou, 1999). This background has stimulated interest in the possibility of antioxidant supplements as a tool to prevent or slow down the progression of such diseases and indeed several investigations have confirmed the potential benefit of this strategy (Silva, Ferreres, Malva, & Dias, 2005). Synthetic antioxidants have been in use as food additives for a long time, but safety concerns and reports on their involvement in chronic diseases have restricted their use in foods. Therefore, international attention has been directed toward natural antioxidants mainly from plant sources (Claudio & Hector, 2000). The objective of this study was to investigate the antioxidant potencies of four different Iranian medicinal plants, namely *Teucrium polium* (TPE), *Cyperus rotundus* (CRE), *Anethum graveolens* (AGE) and *Nasturtium officinale* (NOE), which are currently used in Iranian folk medicine (Table 1). Despite the publication of several ethnobotanical and ethnopharmacological surveys on the therapeutic use of these plants (Bahramikia & Yazdanparast, 2007; Esmaeili & Yazdanparast, 2004; Ljubuncic et al., 2006; Yazdanparast & Ardestani, 2007) (Table 1), laboratory data on the bioactivity of these indigenous medicinal plants against protein oxidation is lacking. We were specifically interested to test if these medicinal plants counteract protein oxidation damages such as formation of PCO, loss of P-SH groups, peroxidation of lipids and generation of ROS caused by Fe^{2+}/ascorbate system.

2. Materials and methods

2.1. Materials

Ascorbic acid, catechin, ferrous sulphate ($FeSO_4$), trichloroacetic acid (TCA), 2,4-dinitrophenylhydrazine (DNPH) and Folin–Ciocalteu's reagent (FCR) were obtained from Sigma (St. Louis, MO). 5,5′-Dithiobisnitrobenzoic acid (DTNB), hydrogen peroxide (H_2O_2), butylated hydroxytoluene (BHT) and thiobarbituric acid (TBA) were obtained from Merck (Darmstadt, Germany). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was purchased from Molecular Probe (Eugene, OR). All other chemicals used were analytical grade.

2.2. Plant material and preparation of extracts

Plants were collected as described in Table 1. The aerial parts of each plant were dried at room temperature away from sunlight. The dried leaves were pulverised and kept at 8 °C for further use. Three hundred grams of the powder of each plant was extracted four times with ethanol (EtOH) (70%), at room temperature overnight. The EtOH extracts were combined and concentrated under reduced pressure on a rotary evaporator, filtered and then lyophilised. The residues were then dissolved in a fixed volume of water (10 mg/ml) and were used in antioxidant measurements. Dose-dependent antioxidant evaluation of each extract indicated that over a concentration range of 25–50 μg/ml each plant extract had a significant antioxidant effect. Therefore, the entire investigation was performed over this concentration range.

2.3. Preparation of liver homogenate and induction of oxidative stress by Fe^{2+}/ascorbate

Male Wistar albino rats weighing 200–250 g (purchased from Pasteur Institute, Tehran, Iran) were housed under conventional conditions and were allowed free access to food and water, *ad libitum*. All experiments were carried out according to the guidelines for the care and use of experimental animals, approved by the state veterinary administration of University of Tehran. The rats were anaesthetised using diethyl ether, their abdomens were opened and their livers were quickly removed. Each liver was then cut into small pieces and homogenised in phosphate buffer (50 mM, pH 7.4), to give a 10% (w/v) liver homogenate. Each homogenate was then centrifuged at 5000*g* for 15 min at 4 °C. The protein concentration of the supernatant was determined by the method of Lowry, Rosebrough, Far, and Randell (1951), using bovine serum albumin as the standard. The oxidant pair Fe^{2+}/ascorbate was used to induce oxidative stress in rat liver homogenate (Ardestani & Yazdanparast, 2007a). The reaction mixture was composed of 0.5 ml of each liver homogenate, 0.9 ml phosphate buffer (50 mM, pH 7.4), 0.25 ml $FeSO_4$ (0.01 mM), 0.25 ml ascorbic acid (0.1 mM), and 0.1 ml of different concentrations of each extract and/or catechin standard. The reaction mixture was incubated at 37 °C for 30 min.

2.4. Determination of protein carbonyl content

Protein carbonyls (PCOs) were measured using the method of Reznick and Packer (1994). One millilitre of 10 mM DNPH in 2 M HCl was added to the reaction mixture (2 mg protein). Samples were incubated for 1 h at room temperature and were vortexed every 15 min. Then, 1 ml of cold trichloroacetic acid (TCA) (10%, w/v) was added to each reaction mixture and centrifuged at 3000*g* for 10 min. The protein pellet was washed three times with 2 ml of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 ml of guanidine hydrochloride (6 M, pH 2.3) and incubated for 10 min at 37 °C whilst mixing. The carbonyl content was calculated, based on the molar extinction coefficient of DNPH ($\varepsilon = 2.2 \times 10^4\ cm^{-1}\ M^{-1}$). The data were expressed in terms of percentage inhibition, calculated from a control measurement of the reaction mixture without the test sample.

2.5. Determination of protein-bound sulphydryl groups

P-SH groups in each liver homogenate were measured according to the methods of Sedlak and Lindsay (1968), using 5,5′-dithi-

obisnitrobenzoic acid (DTNB). For total thiol (T-SH) measurement, an aliquot (0.3 ml) of each liver homogenate was mixed with 1.5 ml of Tris buffer (0.2 M, pH 8.2) and 0.1 ml of 0.01 M DTNB. The mixture was made up to 5.0 ml with 3.1 ml of absolute methanol. After 15 min, each reaction mixture was centrifuged at 3000*g* at room temperature for 15 min. The absorbance of each supernatant was read at 412 nm. For non-protein thiol (NP-SH) measurement, an aliquot of 1.7 ml of each homogenate was mixed with 0.3 ml distilled water and 1 ml of 50% TCA. The tubes were shaken intermittently for 10–15 min and centrifuged for 15 min at 3000*g*. Two millilitres of supernatant were mixed with 2 ml of Tris buffer (0.4 M, pH 8.9), and 0.1 ml DTNB was added. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no liver homogenate. The experimentally determined molar extinction coefficient at 412 nm was 13,100 in both T-SH and NP-SH procedures. The P-SH groups were calculated by subtracting the NP-SH from T-SH and expressed as nmol/mg protein.

2.6. Determination of lipid peroxidation

The extent of lipid peroxidation of the rat liver homogenate in the presence and absence of different plant extracts was evaluated by measuring the product of thiobarbituric acid reactive substances (TBARS) using the method previously described (Ardestani & Yazdanparast, 2007a). After incubation, each reaction was terminated by adding BHT (2% w/v in 95% v/v ethanol) followed by addition of 1 ml of TCA (20% w/v) to the mixture. After centrifugation at 3000*g* for 15 min, the supernatant was incubated with 1 ml of thiobarbituric acid (TBA) (0.67%) at 100 °C for 15 min. The colour intensity of the TBARS/TBA complex was measured at 532 nm. The amount of TBARS formed was calculated using the absorption coefficient of $1.56 \times 10^5\ cm^{-1}\ M^{-1}$. The data were expressed in terms of percentage inhibition, calculated from a control measurement of the reaction mixture without the test sample.

2.7. Measurement of ROS levels

The extent of ROS formation in the reaction mixture was measured by following the oxidation of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) to the highly fluorescent compound, 2′,7′-dichlorofluorescein (DCF), according to a previously published method with slight modification (Ugochukwu & Cobourne, 2003). Each sample contained 1.85 ml of phosphate buffer (50 mM, pH 7.4) solution, 0.1 ml of liver homogenate, and 50 μl of DCFH-DA solution (10 μM). The samples were incubated in a warm water bath at 37 °C for 15 min. The ROS concentrations were measured *via* the formation of DCF using a spectrofluorometer (model Cary Eclipse; Varian Inc., Palo Alto, CA) with the excitation and emission wavelengths at 488 and 525 nm, respectively.

2.8. Determination of total phenolic content

Total phenolic contents were determined with the Folin–Ciocalteu's reagent (FCR), according to a published method (Slinkard & Singleton, 1977). Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v), followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30 °C for 90 min. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extracts).

2.9. Determination of total flavonoid content

The total flavonoid contents were evaluated by a colorimetric method, as described in the literature (Zhishen, Mengcheng, & Jianming, 1999). Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%). After 6 min, 0.15 ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) were added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm *versus* a water blank. Results were expressed as catechin equivalents (mg catechin/g dried extract).

2.10. Statistical analyses

All data are presented as means ± S.D. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents. Statistical analyses were performed using student's *t*-test. Statistical significance was achieved when $p < 0.05$.

3. Results and discussion

3.1. Inhibitory effects on PCO formation

Fe^{2+}/ascorbate model is a well-validated system for production of ROS. A combination of ascorbate and iron can trigger a Fenton reaction with formation of highly reactive hydroxyl radicals which can cause chain-initiation reaction of lipid peroxidation or trigger complex pathways of protein oxidation. In the above model, proteins are known to be damaged by ROS directly and to be targets of secondary modifications by aldehydic products of lipid peroxidation or ascorbate autoxidation. All these processes can collectively result in carbonyl modification of proteins. The assessment of PCO content is a widely-used marker for oxidative protein modification (Reznick & Packer, 1994). Fig. 1 demonstrates the enhanced formation of PCO in the presence of Fe^{2+}/ascorbate. The addition of the oxidant pair to the liver homogenate significantly increased the extent of PCO formation, compared to the control sample. However, in the presence of each of the plant extracts at two different concentrations of 25 and 50 μg/ml, the extent of PCO was significantly reduced. The results were compared with catechin (Cat) as the positive control. Simultaneous addition of Fe^{2+}/ascorbate and catechin resulted in a higher inhibitory effect on PCO formation, relative to the plant extracts as indicated in Fig. 1. Based on Fig. 1, the order of inhibition of PCO formation by the four extracts is: TPE > CRE > AGE > NOE. The principles of protein modification by ROS are well established, as are the char-

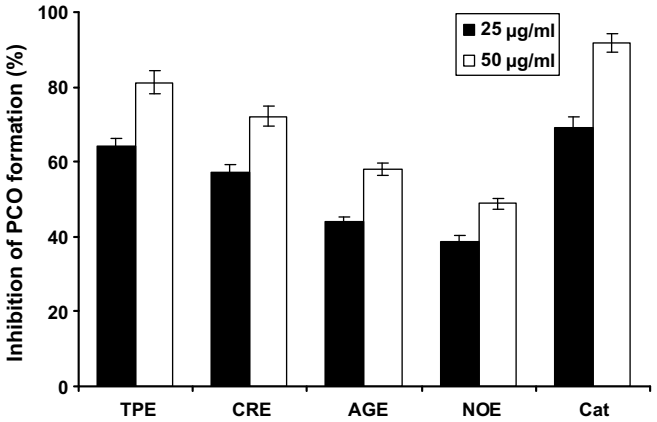

Fig. 1. Inhibitory effects of *T. polium* extract (TPE), *C. rotundus* extract (CRE), *A. graveolens* extract (AGE), *N. officinale* extract (NOE) and catechin against protein carbonyl (PCO) formation induced by Fe^{2+} ascorbate system. Each value represents the mean ± SD ($n = 3$).

acterised reaction products of protein interactions with O_2^- and ·OH. Oxidative damage to several amino acid residues and/or the peptide backbone of proteins can generate PCO products. The oxidation of lysine, arginine, proline and threonine residues may yield carbonyl derivatives (Dean et al., 1997; Stadtman & Levin, 2000). Ascorbate is a known reducing agent, used classically to reduce transition metals, such as Fe^{3+} or Cu^{2+}, and to generate hydroxyl radicals (Buettner, 1986). Furthermore, the ability of transition metals to catalyse autoxidation of ascorbate and therefore to promote protein damage has been reported. The ene-diol anion of ascorbate is very susceptible to autoxidation, *via* metal ions, and produces superoxide anions (Marx & Chevion, 1986). Accordingly, the inhibitory effects of medicinal plants, especially TPE and CRE, on protein oxidation might operate in one or more of the following ways: production of reactive aldehyde scavengers, formation of potential complexes of pro-oxidant metals and scavenging of hydroxyl radicals. It has been shown that TPE and CRE possess potent antioxidants with high scavenging activities against various species of free radicals, such as superoxides and hydroxyl radicals, and the capacity to chelate iron ions (Ljubuncic et al., 2006; Yazdanparast & Ardestani, 2007). Recently, we have unequivocally established that TPE and CRE can effectively inhibit glycoxidation processes (Ardestani & Yazdanparast, 2007b; Ardestani & Yazdanparast, 2007c). The data presented herein provide additional evidence that TPE and CRE suppress oxidative modification of proteins. In that respect, TPE was as potent as catechin in protecting against PCO formation in the rat liver homogenate model.

Protein carbonylation has been associated with important functional alterations in a variety of structural proteins and enzymes. For example, actin carbonylation is a sign of severe functional impairment associated with filament disruption, and occurs at an extent of oxidative damage observed in Alzheimer's disease, inflammatory bowel disease and rat myocardial ischaemia (Dalle-Donne et al., 2003). The results clearly indicate the importance of medicinal plants as potential sources for different antioxidant substances. Since natural antioxidants are relatively non-toxic, the present findings suggest that some of these natural components might be useful in preventing oxidative damage under various pathological conditions.

3.2. Inhibitory effects on P-SH oxidation

P-SH groups are very susceptible to oxidation by free radicals (Stadtman & Levin, 2000).The antioxidant function of P-SH groups might be due to their scavenging activity, protecting cellular constituents against free radical attacks (Telci et al., 2000). Therefore, the measurement of sulphydryl groups in proteins is a useful approach for monitoring the oxidative state of a biological system. Fig. 2 shows the changes in the sulphydryl content of proteins in rat liver homogenates induced by Fe^{2+}/ascorbate system in the presence of the plant extracts. Addition of Fe^{2+}/ascorbate to a reaction reduced the decrease in P-SH contents, compared to the control sample. However, in the presence of each of the plant extracts, at two concentrations (25 and 50 μg/ml), the P-SH content increased to various degrees. Among the four plants, the highest protection level was due to TPE and the lowest value observed for NOE, indicating higher potency of TPE, compared to the other plants. Catechin, as a positive control, resulted in a sharp rise in P-SH levels. Based on thiol–antioxidant activity, the extracts have the following ranking order: TPE > CRE > AGE > NOE. The antioxidant activity of the plant extracts can be due to chelation of redox active metals, as well as to the trapping of some ROS. We have recently found that TPE can effectively inhibit oxidative processes, mainly GSH oxidation in streptozotocin-induced oxidative stress models of diabetes (unpublished data). Consequently, consumption of antioxidants under the oxidative stress state seems crucial for protecting the functional sulphydryl groups of proteins

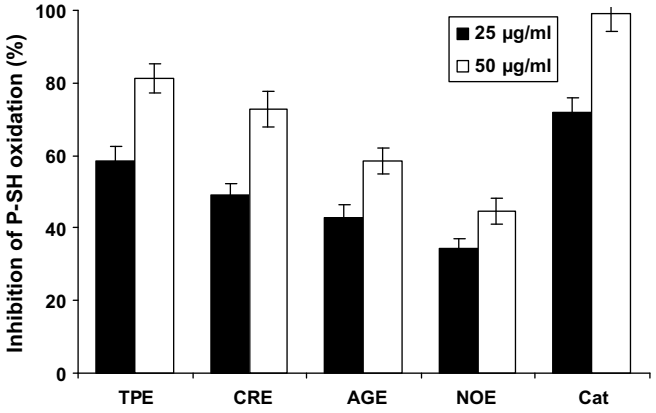

Fig. 2. Inhibitory effects of *T. polium* extract (TPE), *C. rotundus* extract (CRE), *A. graveolens* extract (AGE), *N. officinale* extract (NOE) and catechin against protein thiol (P-SH) oxidation induced by Fe^{2+} ascorbate system. Each value represents the mean ± SD (n = 3).

3.3. Inhibitory effects on lipid peroxidation

The Fe^{2+}/ascorbate model is a well-established system for investigating *in vivo* and *in vitro* lipid peroxidation. On the other hand, in crude tissue fractions incubated *in vitro*, Fe^{2+} can degrade lipid hydroperoxides, forming radicals that can catalyse the chain propagation phase of lipid peroxidation without involving directly the ·OH or other active oxygen species (Dean et al., 1997). In this study, we measured the potential of each of the plant extracts to inhibit lipid peroxidation in the rat liver homogenate induced by the Fe^{2+}/ascorbate system. Fig. 3 demonstrates oxidative damage measured in terms of TBARS. The addition of Fe^{2+}/ ascorbate to the liver homogenate for 30 min significantly increased the extent of TBARS formation relative to the control sample. However, as shown in Fig. 3, addition of two different concentrations of each plant extract (25 and 50 μg/ml) to the liver homogenates of rats significantly reduced TBARS values by varying degrees. The highest percent of inhibition was found for TPE, followed by CRE and AGE, and the lowest activity was found in NOE. As the positive control, catechin showed a high inhibitory effect, compared to the plant

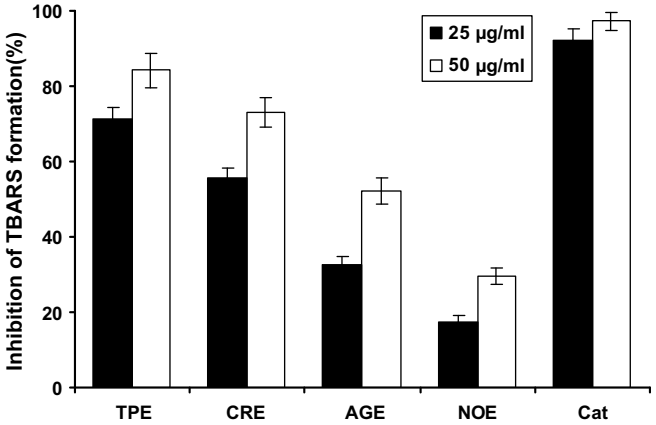

Fig. 3. Inhibitory effects of *T. polium* extract (TPE), *C. rotundus* extract (CRE), *A. graveolens* extract (AGE), *N. officinale* extract (NOE) and catechin against lipid peroxidation induced by Fe^{2+} ascorbate system. Each value represents the mean ± SD (n = 3).

extracts. There is increasing evidence that oxidative modification of proteins also occurs by reactions with aldehydes produced during lipid peroxidation (Traverso et al., 2004). In that regard, end products of lipid peroxidation, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) as well as products from polyunsaturated fatty acids cause protein damage. MDA, 4-HNE and other lipid peroxidation products can oxidise protein thiols or incorporate carbonyl groups into polypeptide chains or cause covalent cross-linking of different protein molecules or fragmentation of polypeptide chains, leading to impairment of protein function (Dean et al., 1997; Traverso et al., 2004). In this study, induction of oxidative stress in rat liver homogenate uniformly resulted in an increase in lipid peroxidation levels. However, treatment with the plant extracts significantly decreased TBARS contents. Consequently, it can be concluded that plant extracts, by decreasing lipid peroxidation, may be effective in preventing oxidative protein damage which is believed to occur during oxidation processes.

3.4. Inhibitory effects against ROS formation

Exposure to Fe^{2+}/ascorbate can transform the redox state to a more oxidising environment and elevate ROS levels. Many previous studies have shown increased oxidative status in various samples exposed to this oxidant system, based on direct determination of either ROS levels or the oxidation end products (Ardestani & Yazdanparast, 2007a). Thus, measurement of ROS level has the potential not only to determine the extent of oxidative injury, but also to predict the potential efficiency of plant extracts aimed at reducing the oxidative stress. ROS formation was analysed by following the increase in DCF fluorescence upon exposure to Fe^{2+}/ascorbate. Fig. 4 shows that oxidative stress induced a significant increase in the fluorescence intensity of DCF in the presence of oxidant pairs, as compared to the control. Incubation of each of the plant extracts and/or catechin at the 25 and 50 μg/ml concentrations reduced Fe^{2+}/ascorbate-induced DCF fluorescence (Fig. 4). These data suggest the potential capacity of the plant extracts to reduce basal ROS production in liver homogenate under oxidative condition. Although, the effects of the plant extracts as well as catechin in reducing DCF fluorescence were mainly associated with a decrease in the level of ROS, we cannot exclude the possibility that the plant extracts as well as catechin can scavenge the DCF semiquinone free radical intermediates (oxygen radical) produced during the formation of the fluorescent product DCF. The order of antioxidant activity as determined by DCF fluorescence (TPE > CRE > AGE > NOE) was in accordance with the inhibition of PCO formation, loss of P-SH and TBARS formation.

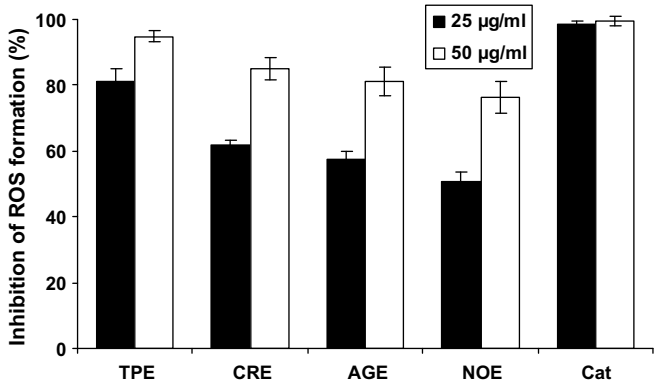

Fig. 4. Inhibitory effects of *T. polium* extract (TPE), *C. rotundus* extract (CRE), *A. graveolens* extract (AGE), *N. officinale* extract (NOE) and catechin against reactive oxygen species (ROS) formation induced by Fe^{2+} ascorbate system. Each value represents the mean ± SD (n = 3).

3.5. Total polyphenolic contents

There is currently an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. These natural antioxidants not only protect food lipids from oxidation, but may also provide health benefits associated with preventing damage due to biological degeneration. The aim is to use them in foods and pharmaceutical preparations to replace synthetic antioxidants. The results of recent research clearly indicate the importance of fruit, vegetable and medicinal plants as the richest potential sources for different antioxidant substances. Independent studies have shown that higher fruit and vegetable consumption is associated with a decreased incidence of cardiovascular diseases, cancer and other chronic diseases (Mozaffarian et al., 2003). The beneficial effects of fruits and vegetables have been attributed, in part, to polyphenolic compounds, especially flavonoids. Flavonoids are a class of secondary plant phenolics found ubiquitously in fruits and vegetables, as well as food products, which act as pharmacological active compounds in many medicinal plants. Many of the biological actions of flavonoids have been attributed to their powerful antioxidant properties (Ardestani & Yazdanparast, 2007a). Based on various investigations, these compounds interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes. In this study, we measured the total phenolic and flavonoid contents of the four plant extracts by using colorimetric methods. As shown in Table 2, the highest phenolic and flavonoid contents were found in TPE, which might be responsible for its enhanced antioxidative activity, compared to the other plants. The total phenolic content of plant extracts ranged from 78 to 180 mg gallic acid/g dry weight, as shown in Table 2. In addition, the total flavonoid content was also determined in the present study, and was expressed based on mg catechin/g dry weight of each plant extract. The values of the total flavonoids of the four plants varied between 49 and 135 mg of catechin/g dry extract (Table 2). The total phenolic and the total flavonoids contents showed a similar pattern in ranking: TPE followed by AGE, NOE and CRE. The observation that TPE had a higher antioxidant activity against protein oxidation, compared to the other plant extracts, was probably due to its higher polyphenolic content. Balu, Sangeetha, Murali, and Panneerselvam (2005) demonstrated that treatment of aged rats with grape seed extract significantly suppressed PCO levels and increased protein thiol content. The authors suggested that the polyphenolics of the grape seed extracts might function as *in vivo* antioxidants by virtue of their ability to directly scavenge ROS and consequently reduce protein oxidation. The antioxidant capacity of each of the four extracts used in this study might be due to their phenolic and flavonoid contents. However, involvement of other constituents should not be disregarded. In this regard, although the total phenolic and flavonoid content of the *C. rotundus* extract is lower than those of *A. graveolens* and *N. officinale* extracts (Table 2), its corresponding antioxidant activity against protein oxidation, lipid peroxidation and ROS formation

Table 2
Total phenol and total flavonoid contents of four Iranian medicinal plants.

Samples	Total phenol content[a]	Total flavonoids content[b]
T. polium	180.2 ± 7.5	135.2 ± 4.9
C. rotundus	78.5 ± 4.2	43.9 ± 1.7
A. graveolens	105.2 ± 3.2	58.2 ± 2.8
N. officinale	97.2 ± 3.5	49.2 ± 2.4

Each value represents the mean ± SD (n = 3).
[a] Total phenol content was expressed as mg gallic acid/g dried extract.
[b] Total flavonoid content was expressed as mg catechin/g dried extract.

is higher. Exact interpretation of these data awaits full purification and structural elucidation of the responsible antioxidants from each plant extract.

4. Conclusion

The implication of oxidative stress in the aetiology and progression of several acute and chronic clinical disorders has led to the suggestion that antioxidants can have health benefits as prophylactic agents. In recent years, there has been a worldwide trend toward the use of natural phytochemicals present in fruits and vegetables with antioxidant properties and health-promoting phytochemicals. Plant-derived polyphenols display characteristic inhibitory patterns toward the oxidative reaction *in vitro* and *in vivo*. Oxidative damage of proteins is one of the modifications leading to severe failure of their biological functions and even cell death. Oxidative stress and lipid peroxidation are known to mediate a destructive effect on proteins in our model. Herein we report the protective effects of four Iranian medicinal plants against oxidative damages to proteins induced by a free radical generating system. Thus, the suppressive effect of each plant extract on protein oxidation could be attributed to its antioxidant activity, in addition to the suppression of lipid peroxidation. In addition, we show that *T. polium* is more effective than the other plant extracts in protecting against the oxidative damage caused by the Fe^{2+}/ascorbate system.

Acknowledgments

The authors appreciate the joint financial support of this investigation by the Research Council of University of Tehran, and Iran National Science Foundation.

References

Ardestani, A., & Yazdanparast, R. (2007a). Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chemistry, 104*, 21–29.

Ardestani, A., & Yazdanparast, R. (2007b). Inhibitory effects of ethyl acetate extract of *Teucrium polium* on in vitro protein glycoxidation. *Food and Chemical Toxicology, 45*, 2402–2411.

Ardestani, A., & Yazdanparast, R. (2007c). *Cyperus rotundus* suppresses AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation. *International Journal of Biological Macromolecules, 41*, 572–578.

Aruoma, O. I. (1998). Free radical, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society, 75*, 199–212.

Bahramikia, S., & Yazdanparast, R. (2007). Effect of hydroalcoholic extracts of *Nasturtium officinale* leaves on lipid profile in high-fat diet rats. *Journal of Ethnopharmacology, 115*, 116–121.

Balu, M., Sangeetha, P., Murali, G., & Panneerselvam, C. (2005). Age-related oxidative protein damages in central nervous system of rats: Modulatory role of grape seed extract. *International Journal of Developmental Neuroscience, 23*, 501–507.

Buettner, G. R. (1986). Ascorbate autoxidation in the presence of iron and copper chelates. *Free Radical Research Communication, 1*, 349–353.

Chaudiere, J., & Ferrari-Iliou, R. (1999). Intracellular antioxidants: From chemical to biochemical mechanism. *Food and Chemical Toxicology, 37*, 949–962.

Claudio, C., & Hector, F. (2000). Synthetic antioxidants determination in lard and vegetable oils by the use of voltammetric methods on disk ultramicroelectrode. *Food Research International, 33*, 357–365.

Dalle-Donne, I., Giustarini, D., Colombo, R., Rossi, R., & Milzani, A. (2003). Protein carbonylation in human diseases. *Trends in Molecular Medicine, 9*, 169–176.

Dean, R. T., Fu, S., Stocker, R., & Davies, M. J. (1997). Biochemistry and pathology of radical-mediated protein oxidation. *Biochemical Journal, 324*, 1–18.

Esmaeili, M. A., & Yazdanparast, R. (2004). Hypoglycaemic effects of *Teucrium Polium*: Studies with rat pancreatic islets. *Journal of Ethnopharmacology, 95*, 27–30.

Halliwell, B., & Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine* (3rd ed.). Oxford University Press.

Kayali, R., Cakatay, U., & Tekeli, F. (2007). Male rats exhibit higher oxidative protein damage than females of the same chronological age. *Mechanisms of Ageing and Development, 128*, 365–369.

Ljubuncic, P., Dakwar, S., Portnaya, I., Cogan, U., Azaizeh, H., & Bomzon, A. (2006). Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity in vitro. *Evidence-Based Complementary and Alternative Medicine, 3*, 329–338.

Lowry, O. H., Rosebrough, N. J., Far, A. L., & Randell, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Marx, G., & Chevion, M. (1986). Site-specific modification of Albumin by free radicals. Reaction with copper (II) and ascorbate. *Biochemical Journal, 236*, 397–400.

Mozaffarian, D., Kumanyika, S. K., Lemaitre, R. N., Olson, J. L., Burke, G. L., & Siscovick, D. S. (2003). Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. *Journal of American Medical Association, 289*, 1659–1666.

Reznick, A. Z., & Packer, L. (1994). Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. *Methods in Enzymology, 233*, 357–363.

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry, 25*, 192–205.

Silva, B. A., Ferreres, F., Malva, J. O., & Dias, A. C. P. (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chemistry, 90*, 157–167.

Slinkard, J., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Stadtman, E. R., & Levin, R. L. (2000). Protein oxidation. *Annals of the New York Academy of Sciences, 899*, 191–208.

Telci, A., Cakatay, U., Salman, S., Satman, I., & Sivas, A. (2000). Oxidative protein damage in early stage Type 1 diabetic patients. *Diabetes Research and Clinical Practice, 50*, 213–223.

Traverso, N., Menini, S., Maineri, E. P., Patriarca, S., Odetti, P., Cottalasso, D., Marinari, U. M., & Pronzato, M. A. (2004). Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to proteins. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 59*, 890–895.

Ugochukwu, N. H., & Cobourne, M. K. (2003). Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with extracts from *Gongronema latifolium* leaves. *Clinica Chimica Acta, 336*, 73–81.

Yazdanparast, R., & Ardestani, A. (2007). In vitro antioxidant and free radical scavenging activity of *Cyperus rotundus*. *Journal of Medicinal Food, 10*, 667–674.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.