Indian Journal of Experimental Biology
Vol. 47, May 2009, pp. 338-342

Protective effects of *Petroselinum crispum* (Mill) Nyman ex A. W. Hill leaf extract on D-galactose-induced oxidative stress in mouse brain

Shreya R Vora*, Rahul B Patil† & Meena M Pillai††
Department of Zoology, Shivaji University, Kolhapur. 416 004 India

Received 2 June 2008; revised 2 March 2009

With an aim to examine the effect of ethanolic extract of *P. crispum* (Parsley) leaves on the D-galactose-induced oxidative stress in the brain of mouse, the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) involved in oxygen radical (OR)-detoxification and antiperoxidative defense were measured in conjunction with an index of lipid peroxidation in mitochondrial fraction of various regions of the mouse brain. A significant decrease in superoxide dismutase and glutathione peroxidase activity was observed in D-galactose-stressed mice, while catalase activity was increased. Treatment of D-galactose-stressed mice with the ethanolic extract of *P. crispum* showed protection against the induced oxidative stress in brain regions. Concentration of thiobarbituric acid-reactive product was greatly elevated in D-galactose stress-induced mice and was significantly reduced in the brain regions of these mice upon treatment with *P. crispum*. It is postulated that parsley shows a protective effect against mitochondrial oxidative damage in the mouse brain.

Keywords: Catalase, D-galactose, Glutathione peroxidase, Lipid peroxidation, *Petroselinum crispum*, Superoxide dismutase

Oxygen free radicals are reactive species which are constantly generated in living cells as a part of normal metabolism. Mitochondria are the major source of the free radicals in the cells. Under normal physiologic conditions, a small fraction of the oxygen consumed by mitochondria is constantly converted to free radicals like superoxide anions, hydroxyl radical and other reactive oxygen species (ROS). An excess production of ROS is harmful to cells, which is likely to exert toxic effects in the cells involved in the pathogenesis of certain diseases and aging. To scavenge and neutralize these free radicals, the cells are endowed with the antioxidant defense system of enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). But an imbalance between reactive oxygen metabolites and antioxidant defense mechanisms of the cells, leading to excessive production of free radicals, creates a condition termed as oxidative stress[1,2]. The oxidative stress in the cells leads to lipid peroxidation, inactivation of enzyme activities including antioxidant enzymes and DNA breakage[3]. Postmitotic cells especially nerve cells are very susceptible to the oxidative damage due to their high consumption of oxygen and presence of large amounts of fatty acids that are prone to peroxidation. Thus, oxidative damage is evident in a wide range of degenerative diseases and aging[4].

The evidence for the potential role of the oxidants in the pathogenesis of many diseases suggests that antioxidants may be of therapeutic use in these conditions. Polyphenols in plants are a versatile group of antioxidants that protect against oxidative damage by directly neutralizing reactive oxygen species. A large amount of polyphenols is present in asparagus, radish, carrot, onion, beet, cabbage, lettuce and parsley in the form of flavonol glycosides[4].

Parsley [*Petroselinum crispum* (Mill.) Nyman ex A W Hill] (Umbelliferae) is cultivated throughout the world and used as a spice, salad and herbal remedy. Use of parsley in food has a long history

*Correspondant author
Telephone: (0233) 26909249
E-mail: dr_shreyavora@rediffmail.com

Present addresses;

†Government Rajaram College, Kolhapur 416 004, India
rb_aging@rediffmail.com

††Department of Biotechnology Engineering
KIT'S College of Engineering, Kolhapur
mmpillai_gerontol@rediffmail.com

going back to ancients, Greeks and Romans. It has been reported to have possible medicinal attributes as an antioxidative, antimicrobial, anticoagulant, antihyperlipidemic and antihepatotoxic[5]. The parsley leaves are highly nutritious and considered a natural vitamin and mineral source. It contains ascorbic acid, tocopherol, flavonol glycosides of quercetin, apiole, myristicin and luteolin[6,7].

It also possesses terpenes, phthalides, starch, vitamin A, C, E, iron, calcium, phosphorus and manganese. Quercetin and rutin are the most studied flavonoids[8,9], and they have shown potential reactivity with active oxygen species which is the prominent characteristic of flavonoids[10,11]. Quercetin, rutin and myristicin are the most effective inhibitors of superoxide radicals, which initiate the chain reaction of generation of ROS[12].

Though polyphenols are excellent antioxidants, there is an important distinction between the *in vitro* antioxidant effectiveness of an antioxidant and its ability to prevent oxidation *in vivo*. This depends on their absorption, transportation and incorporation into appropriate tissues and cellular sites. It is, therefore important to study the effect of particular flavonoids on a particular tissue and particular cellular site. In this context, antioxidant effects of parsley leaf extract (ethanolic) have been studied on the brain of mouse oxidatively-stressed by using D-galactose-induced aging mouse model[13]. Male albino mice (*Mus musculus*) were subjected to the accelerated aging by injecting a low dose of D-galactose which is a reducing sugar and is capable of reacting with biomolecules in the cells like proteins and lipids without any enzymatic intervention. The reactions are called as Maillard reactions or glycation. The complexes of sugars thus formed are termed as the advanced glycation end products (AGEs). The accumulation of AGEs in cells provokes formation of free radicals which are responsible for the pathogenesis of various diseases and aging as well[14].

Materials and Methods

Animals—Six months old albino male mice (*Mus musculus*) used for the present study were housed in Departmental Animal House approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were kept under a 12:12 hr L:D cycle and fed *ad libitum* a commercial chow diet (Pranav Agro Industries, Sangli India). Animals were randomly assigned to the following three groups. The first group of animals received only sterile water and food *ad libitum*, the second group of animals was injected with 5% D-galactose at a dose of 0.5 ml/day for 20 days[16] and the third group of animals was subcutaneously co administered D-galactose and an ethanolic extract of parsley leaves at a dose of 40 mg/kg (body weight) for 20 days.

Preparation of plant extract—Properly identified fresh parsley plant was obtained from the garden. Fresh leaves were separated, washed and rinsed with distilled water and dried in shade and then crushed, powdered and soaked in distilled alcohol for 72 hr. The alcohol was evaporated by using high-speed vacuum evaporator (Buchi type) to obtain a thick paste-like extract. The extract was stored in glass bottle at 4°C for further use.

Preparation of homogenate—The animals were sacrificed by cervical dislocation after completion of respective doses. The brain was excised out by removing cranium and taken on a prechilled petri plate. Cerebellum was identified just above the medulla. Four lobed corpora qaudrigemina was identified between cerebellum and cerebral lobes. To separate cerebral cortex and hippocampus, a superficial and perpendicular cut to the axis of brain was made in the cortex region where the two lobes of the cerebrum demarcate. The upper mass of cerebral cortex was removed delicately with pointed forceps. Below this, horse-shoe shaped hippocampus was seen. Thus, cerebral cortex and hippocampus were separated. These brain tissues i.e. cerebral cortex, hippocampus, corpora qaudrigemina and cerebellum were frozen and thawed. The homogenates were prepared in 0.25 *M* sucrose and 1 m*M* EDTA using Teflon homogenizer at 4°C. The homogenates were centrifuged at 1000 g for 15 min at 4°C to remove cell debris and nuclear fractions. The supernatants were then centrifuged at 16,900 g for 20 min at 4°C and the supernatants 'A' and pellets 'B' thus obtained were used for estimations as follows.

Enzyme activity assay—The supernatant 'A' was used for the estimation of catalase[15] and glutathione peroxidase[16]. The pellet 'B' was suspended in phosphate buffer (0.1 *M*, *p*H 7.8) and was treated as enzyme source for the assay of superoxide dismutase[17]. The enzyme activities were expressed as unit enzyme activity/mg protein.

Lipid peroxidation assay—The extent of lipid peroxidation in the form of malondialdehyde (MDA)

was determined by the method of Wills[18]. The pellet 'B' was suspended in 20% triton X-100 and again centrifuged at 16900 g for 20 min at 4°C. The pellet thus obtained was suspended in a reaction mixture containing phosphate buffer 75 m*M*, *p*H 7.04, ascorbic acid 1 m*M* and ferric chloride 1 mM and treated with 0.67% thiobarbituric acid (TBA) to develop pink colour of MDA. The developed colour was measured on spectrophotometer at 532 nm and expressed as n mol MDA/mg wet tissue.

Protein analysis—Protein concentrations in the tissue homogenates were determined using the method of Lowry *et al*[19] using bovine serum albumin as the standard.

Statistical analysis—Statistical analysis was performed by Student's *t*-test. $P<0.001$ is considered highly significant.

Results

Concentration of MDA in various brain regions of D-galactose-stressed group was elevated as compared to that of control ones (Table 1). In the animals which received parsley extract alongwith D-galactose, MDA level was significantly low in all the regions of brain as compared to the D-galactose-treated mice group. The maximum fall in the level of MDA was observed in the cerebral cortex.

The activities of antioxidative defense enzymes in various brain regions of control, galactose-stressed, and parsley plus galactose-treated mice are shown in Tables 1. A decrease in superoxide dismutase and glutathione peroxidase and increase in catalase activity was observed in the brain of D-galactose-stressed mice as compared to the control group. On treatment with ethanolic extract of parsley there was a significant increase in the activities of superoxide dismutase and glutathione peroxidase with decrease in catalase when compared to D-galactose-stressed group.

Discussion

D-galactose is normally present in the body but when its level increases above the normal, it gets oxidized into aldehydes and hydrogen peroxide[20], and stimulates diabetes mellitus, induces premature aging with increased serum AGE content and decreases motor activity[13]. It has also been shown that D-galactose reduces immune responses, and increases oxidative stress by increasing lipid peroxidation, and decreases antioxidant enzyme activities and mitochondrial function by inducing degeneration[21]. Data derived from the present experiments showed marked elevations of lipid peroxidation in mitochondrial fractions, and significant alterations in

Table 1—Effect of ethanolic extract of *P. crispum* leaves on (A) mitochondrial peroxidation and activities of superoxide dismutase (B), catalase (C) and glutathion peroxidase (D) in D-galactose-stressed mice brain
[Values are mean ± SD from 5 animals in each group]

Organ		Normal (Control)	D-galactose-stressed	D-galactose + parsley extract
Cerebral cortex	A	6.823 ± 0.074	10.631 ± 0.307	1.730 ± 0.005
	B	20.676 ± 0.874	6.012 ± 1.754	20.71 ± 1.496
	C	0.079 ± 0.002	0.225 ± 0.004	0.053 ± 0.004
	D	0.034 ± 0.002	0.029 ± 0.0007	0.031 ± 0.0007
Hippocampus	A	4.467 ± 0.216	9.426 ± 0.168	2.413 ± 0.071
	B	22.954 ± 0.783	10.5 ± 1.671	21.35 ± 1.221
	C	0.213 ± 0.004	0.241 ± 0.006	0.108 ± 0.004
	D	0.095 ± 0.003	0.087 ± 0.001	0.093 ± 0.0007
Cerebellum	A	5.094 ± 0.071	6.852 ± 0.063	1.776 ± 0.059
	B	21.434 ± 0.997	9.161 ± 1.598	18.326 ± 0.926
	C	0.315 ± 0.004	0.480 ± 0.006	0.320 ± 0.004
	D	0.090 ± 0.0007	0.080 ± 0.001	0.090 ± 0.0007
Corpora quadrigemina	A	9.189 ± 0.093	10.180 ± 0.164	1.166 ± 0.012
	B	20.31 ± 0.961	7.478 ± 2.347	18.362 ± 2.058
	C	0.182 ± 0.005	0.466 ± 0.006	0.160 ± 0.005
	D	0.073 ± 0.001	0.063 ± 0.001	0.071 ± 0.001

A: nmol MDA/mg tissue; B, C, D: enzyme activity unit/mg protein
P value: < 0.001
All values of D-galactose-stressed group and D-galactose + parsley treated group are highly significant with respect to normal (control) and D-galactose-stressed group respectively

the activities of antioxidant enzymes of various brain regions in D-galactose-stressed mice as compared to the controls. It clearly indicates high oxidative stress in brain regions of D-galactose-stressed mice. Increased oxidative stress in the cells has often been shown to cause alterations in antioxidant enzymes[22]. The highly reactive free radicals generated during oxidative phosphorylation in mitochondria, attack membrane bound lipids, proteins and nucleic acids. This brings about negative impact on mitochondrial metabolism resulting progressive decrease in the efficiency of mitochondrial enzyme system and antioxidant defense system as well. These alterations are very prominent in the brain[23] because it has very poor natural antioxidant defense system[24].

It is noteworthy that though the MDA level was significantly increased in brain regions of D-galactose-stressed mice, mice treated with ethanolic extract of parsley showed no such alterations as compared to controls. Parsley leaf extract significantly elevated the antioxidant ability of the brain tissues of D-galactose-stressed mice. Thus, the present results suggest that parsley has the potential to reduce the formation of TBA-reactive products and elevate the levels of antioxidant enzymes. Ethanolic extract of parsley leaves contains tannins, flavonoids, sterols and triterpenes[25]. The results of the present study showed that ethanolic extract of parsley leaves helped in maintaining the balance of oxidants and antioxidant defense enzyme activities in the brain of mice under D-galactose-induced stress condition.

In sum, the results reveal that administration of ethanolic extract of parsley leaves in D-galactose-stressed mice helps to significantly reduce the levels of lipid peroxidation products and elevate antioxidant enzymes in brain regions.

References

1 Donato H J & Sohal R S, Lipofuscin, in *Handbook of biochemistry of aging*, edited by J R Florini, Rc Adelman and GS Roth (CRC Press, Boca Raton, Florida) 1981, 221.

2 Schroeder F, Role of membrane lipid asymmetry in aging, *Neurobiol Aging*, 5 (1984) 323.

3 Wiseman H & Halliwell B, Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer, *Biochem J*, 313 (1996) 17.

4 Ames B N, Shigenaga M K & Hagen T M, Oxidants, antioxidants and the degenerative diseases of aging, *Proc Natl Acad Sci USA*, 90 (1993) 7915.

5 Yanardag R, Bolkent S, Tabakoglu-Oguz A & Ozsoy-Sacan O, Effects of *Petroselinum crispum* extracts on pancreatic B cells and blood glucose of streptozotocin-induced diabetic rats, *Biol Pharm Bull*, 26 (2003) 1206.

6 Kreuzaler F & Hahlbrock K, Flavonol glycosides from illuminated cell suspension cultures of *Petroselinum hortense*, *Phytochemistry*, 12 (1973) 1149.

7 Chenard C H, Kopsell D A & Kopsell D E, Nitrogen concentration affects nutrient and carotinoids accumulation in parsley, *Plant Nutrition*, 28 (2005) 285.

8 Duthie S J & Dobson V L, Dietary flavonoids protect human colonocyte DNA from oxidative attack *in vitro*, *Eur J Nutr*, 38 (1999) 28.

9 Russo A, Izzo A A, Borrelli F, Renis M & Vanella A, Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage, *Phytother Res*, 17 (2003) 870.

10 Rice-Evans C A, Miller N J, Bolwell P G, Bramley P M & Pridham J B, The relative antioxidant activities of plant derived polyphenolic flavonoids, *Free Radic Res*, 22 (1995) 375.

11 Saija A, Scalese M, Lanza M, Marzullo D, Bonina F & Eabtelli F, Flavonoids as antioxidant agents: importance of their interaction with biomembranes, *Free Radic Bio Med*, 19 (1995) 481.

12 Robak J & Gryglowski R J, Flavonoids are scavengers of superoxide anions, *Biochem Pharmacol*, 37 (1988) 831.

13 Song X, Bao M, Li D & Li Y M, Advanced glycation in D-Galactose induced mouse aging model, *Mech Ageing Dev*, 108 (1999) 239.

14 Hamada Y, Araki N, Kou N, Nakamura J, Horiuchi S & Hotta N, Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway, *Biochem Biophys Res Commun*, 228 (1996) 539.

15 Luck H, *Methods in enzymatic analysis*, second ed., Bergmeyer (Academic Press New York) 1974, 885.

16 Beers R & Sizer I, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, *J Biol Chem*, 195 (1952) 133.

17 Beauchamp C & Fridovich I, Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Anal Biochem*, 44 (1971) 276.

18 Wills E D & Wilkinson A E, Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. *Biochem J*, 99 (1966) 667.

19 Lowry O H, Rosenbrough N J, Farr A L & Randall R J, Protein measurement with folin phenol reagent, *J Biol Chem*, 193 (1951) 265.

20 Ho S C, Liu J H & Wu R Y, Establishment of mimetic aging effect in mice caused by D-galactose, *Biogerontology*, 4 (2003) 15.

21 Ida H, Ishibashi K, Reiser K, Hjelmeland L M & Handa J T, Ultrastuctural aging of the RPE-Bruch's membrane-choriocapillaris complex in the D-galactose-treated mouse, *Investigative Ophthalmol Visual Sci*, 45 (2004) 2348.

22 Skaper S D, Fabris M, Ferrari V, Carbonare M D & Leon A, Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathion depletion: Cooperative effects of ascorbic acid, *Free Radic Biol Med*, 22 (1997) 669.

23 Bourre J M, Protection against peroxidation by radicals in cerebral capillaries and microvessels in aging, edited by Packer L, Pirilipko Y and Christen A, in *Free radicals in the brain: Aging, Neurological and mental disorder* (Springer-Verlag, Berlin, Heidelberg) 1992, 41.

24 Halliwell B, Oxidants and the central nervous system: Some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke?, *Acta Neurol Scand Suppl*, 126 (1989) 23.

25 Al- Howiriny T, Al-Sohaibani M, El-Tahir K & Rafatullah S, Prevention of experimentally-induced gastric ulcer in rats by ethanolic extract of parsley *Petroselinum crispum*, *Am Chinese Med (AJCM)*, 31 (2003) 699.