Mol Cell Biochem
DOI 10.1007/s11010-016-2754-6

Age-related changes in the brain antioxidant status: modulation by dietary supplementation of *Decalepis hamiltonii* and physical exercise

Tekupalli Ravikiran[1] · Ramachandregowda Sowbhagya[1] · Sindhghatta Kariyappa Anupama[1] · Santosh Anand[1] · Dundaiah Bhagyalakshmi[1]

Received: 15 March 2016 / Accepted: 21 June 2016

Abstract The synergistic effects of physical exercise and diet have profound benefits on brain function. The present study was aimed to determine the effects of exercise and *Decalepis hamiltonii* (*Dh*) on age-related responses on the antioxidant status in discrete regions of rat brain. Male *Wistar* albino rats of 4 and 18 months old were orally supplemented with *Dh* extract and swim trained at 3 % intensity for 30 min/day, 5 days/week, for a period of 30 days. Supplementation of 100 mg *Dh* aqueous extract/kg body weight and its combination with exercise significantly elevated the antioxidant enzyme activities irrespective of age. Age-related and region-specific changes were observed in superoxide levels, and protein carbonyl and malondialdehyde contents, and were found to be decreased in both trained and supplemented groups. Levels of total thiols, protein, and nonprotein thiols decreased with age and significantly increased in the SW-T(+100 mg) groups. Our results demonstrated that the interactive effects of two treatments enhanced the antioxidant status and decreased the risk of protein and lipid oxidation in the rat brain.

Keywords *Decalepis hamiltonii* · Swimming training · Antioxidant enzymes · Oxidative markers

Introduction

Oxidative stress is a biological phenomenon associated with a range of degenerative diseases observed during aging [1]. Age-related deficits may be associated with structural and functional changes in macromolecules and cell membranes, which, at least in part, result from direct or indirect effect of free radicals and reactive oxygen species (ROS) [2]. Proteins are the biomolecules which are prone to oxidation wherein they react with free radicals, causing modification of several amino acids, protein aggregation, and fragmentation. Oxidative damage to protein is reflected by markers of oxidative stress such as protein carbonyls and protein thiols. Protein carbonylation is the most common oxidative modification seen with accelerating age [3] which can be assessed by measuring the carbonyl levels. Thiol groups (–SH) play a prominent role in antioxidant reactions, catalysis, regulation, electron transport, and those preserving the correct structure of proteins [4]. Thus, oxidative modification of proteins in vivo may affect a variety of cellular functions involving proteins, receptors, signal transduction mechanisms, transport systems, and enzymes.

The brain is highly susceptible to oxidative stress due to high concentrations of oxidizable substrates, a high rate of oxidative metabolic activity, and endogenous ROS production caused by dopamine oxidation. In addition, it is known that certain regions of the brain are enriched in iron, a metal that is catalytically involved in the production of ROS in living tissues.

Regular physical exercise is an efficient strategy to protect the brain functions against deficits associated with the aging process [5]. Studies in humans [6, 7] and in rodents [8, 9] have demonstrated the beneficial effects of exercise on cognitive function. Previous studies by Devi and Kiran [10] suggests that exercise increases antioxidant enzyme activity and attenuates oxidative stress in brain.

Diet also plays a significant role in brain function [11]. Polyphenols are of great interest in nutrition and medicine

✉ Tekupalli Ravikiran
ravikiran@bub.ernet.in

[1] Department of Biotechnology, Jnana Bharathi Campus, Bangalore University, Bengaluru 560 056, India

Published online: 05 July 2016

because of their potent antioxidant capacity [12]. *Decalepis hamiltonii* (*Dh*), commonly known as swallow root is a climbing shrub and belongs to the family of Asclepiadaceae. The roots are used in folk medicine and ayurvedic preparation as general vitalizer and blood purifier [13]. The aqueous root extract is a cocktail of many polyphenolic compounds which include ellagic acid, 4-hydroxyisophthalic acid, 14-aminotetradecanoic acid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1,2-cyclohexanediol, 2-hydroxymethyl-3-methoxybenzaldehyde, and 2,4,8-trihydroxybicyclo[3.2.1]octan-3-one [14]. Different studies have shown that root extract possesses hepatoprotective, neuroprotective, and cardioprotective activities [13–15].

Studies have shown that synergistic effects of exercise and dietary supplementation have positive effects by attenuating the oxidative stress in the aging rat brain [16, 17]. Therefore, the present study was hypothesized to investigate the combined effects of exercise and *Dh* supplementation in curtailing oxidative stress in the aging rat brain. The hypothesis was tested by analyzing detoxifying antioxidant enzyme activities and markers of oxidative stress as a function of age, exercise, and *Dh* supplementation in the cerebral cortex (CC), hippocampus (HC), and cerebellum (CB) regions of the brain.

Materials and methods

Chemicals

Epinephrine, reduced glutathione, glutathione reductase, thiobarbituric acid, t-peroxyhydroxide, and guanidine hydrochloride were procured from Sigma (St. Louis, MO). All other chemicals that were of analytical grade and solvents that were of spectral grade were procured from Himedia chemicals (Mumbai).

Preparation of Aqueous extract

Tuberous roots of *Dh* were collected from Savandurga forest, Bengaluru, India. The plant material was identified and deposited in the herbarium of the Department of Botany, Bangalore University. The roots were thoroughly washed with water. The inner woody core was separated from outer fleshy layer. The fleshy layer was dried at 40 °C in a hot air oven and fine powdered. The aqueous extract was prepared by dissolving the powder in warm water (50 °C) and allowing it to stand for 24 h, and filtered through Whatman no. 1 paper. The filtrate was lyophilized and weighed [14].

Animal maintenance

All animal procedures were approved by the Institutional Animal Ethics Committee (IAEC), Bangalore University, Bengaluru, India. The study comprised 60 male albino rats of *Wistar* strain aged 4 and 18 months (adult and old). Initially, rats aged 3 months (mo) were obtained from the Central Animal Facility, IISc, Bengaluru. Animals were housed three per cage, in polypropylene fitted steel mesh-bottom cages, and were maintained at a temperature of 28 ± 1 °C, relative humidity of 77 ± 1 % and under a 12-h dark and light cycle. All animals had free access to food (Amruth Feeds, India) and tap water ad libitum. The rats were segregated into three swim exercise trainee groups ($n = 15$)—(i) swim trainees on a normal diet [SW-T(N)], (ii) swim trainees on a daily oral supplementation of *Dh* extract [50 mg/kg BW (SW-T(+Dh_1))], and (iii) swim trainees on a daily oral supplementation of *Dh* extract [100 mg/kg BW (SW-T(+Dh_2))]—and three sedentary groups ($n = 15$)—(i) sedentary on a normal diet [SE-C(N)], (ii) sedentary with a daily *Dh* extract (50 mg/kg BW) supplementation [SE-C(+Dh_1)], and (iii) sedentary with a daily *Dh* extract (100 mg/kg BW) supplementation [SE-C(+Dh_2)].

Swimming training protocol

Swimming exercise training was similar to our earlier protocols with minor modifications [10]. Briefly, rats were swim trained in a rectangular glass tank ($77 \times 38 \times 39$ cm^3) filled with water to a height of 18 cm for young groups and 24 cm for old groups at 32 ± 1 °C with a load of 3 % of their body weight tied to their tails. Initially, they were made to exercise for 5 min/day with a progressive increase to 30 min/day over a period of 1 week, and thereafter for 30 min/day, for a total training period of 30 days with 5 days/week.

Tissue preparation

After the completion of the experiment protocol, the animals were sacrificed under diethyl anesthesia. The brain tissue was excised and the cerebral cortex (CC), hippocampus (HC), and cerebellum (CB) regions were separated, weighed, and homogenized in ice cold 50 mM phosphate buffer (pH 7.0). The homogenate was used for MDA, superoxide radical assays, and estimation of thiols. The homogenate was centrifuged (Plastocrafts, Superspin-RV/FM) at 1000 g at 4 °C for 10 min. The supernatant obtained was used for antioxidant enzyme assays and protein carbonyl estimation.

Measurement of Antioxidant enzyme activities

Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich [18]. Briefly, tissue supernatant (100 μL) was added to 880 μL carbonate buffer of 0.05 M, pH 10.2, and 0.l mM EDTA. 20 μL of 30 mM epinephrine in 0.05 % acetic acid was added to the mixture, and absorbance was followed for 5 min at 480 nm in a spectrophotometer (Model SL 159, ELICO). The amount of enzyme that results in 50 % inhibition of epinephrine auto-oxidation was defined as one unit.

Catalase (CAT) activity was measured according to the method of Aebi [19]. Briefly, 100 μL of the tissue supernatant with an equal volume of absolute alcohol was incubated for 30 min at 0–8 °C following which triton X-100 was added. A known volume of this was taken in an equal volume of 0.066 M H_2O_2 in phosphate buffer and the decrease in absorbance was measured at 240 nm for a minute in a spectrophotometer. An extinction coefficient of 43.6 M cm^{-1} was used to determine enzyme activity, one unit of which was equal to the moles of H_2O_2 degraded/min/mg of protein.

Glutathione peroxidase (GPx) activity was measured at 37 °C by the method of Flohe and Gunzler [20]. Briefly, the reaction mixture consisted of 500 μL of phosphate buffer, 100 μL of 0.01 M reduced glutathione (GSH), 100 μL of 1.5 mM NADPH, and 100 μL of glutathione reductase. One hundred microliters of tissue extract was added to the reaction mixture and incubated at 37 °C for 10 min. Fifty microliters of 12 mM *t*-butyl hydroperoxide was added to 450 μL of tissue reaction mixture and measured at 340 nm for 180 s in a spectrophotometer. A molar absorptivity of 6.22×10^3 M cm^{-1} was used to determine enzyme activity. One unit of activity is equal to mM NADPH oxidized per minute per mg protein.

Measurement of Superoxide radical

Superoxide radicals (SOR) were measured according to the method of Das et al. [21]. Briefly, 200 μL of homogenate was incubated with 80 μL of 0.1 % nitroblue tetrazolium (NBT) in an oscillating water bath for 1 h at 37 °C. Termination of the assay and extraction of the reduced NBT was carried out by centrifuging the samples for 10 min at 200 g then resuspending the pellets with 1 mL of glacial acetic acid. The absorbance was measured at 560 nm and converted to μmoles diformazan using a standard curve generated from nitroblue diformazan (NBD). Final results were expressed as μmoles diformazan/mg tissue.

Measurement of Protein oxidation

Protein carbonyls were measured according to the procedure of Levine et al. [22]. Briefly, 100 μL of supernatant tissue extract was incubated with 0.5 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl for 60 min in dark. Protein was precipitated using 0.5 mL of 20 % TCA and then centrifuged at 10,000×*g* for 3 min at 4 °C. The supernatant was discarded, and the pellet was washed with 1:1 ethyl acetate/ethanol twice by centrifuge method (3400×*g* for 5 min) to remove DNPH. The pellet was dissolved after washing in 1.5 mL of 6 M Guanidine hydrochloride in phosphate buffer (pH 6.5). Absorption was read at 370 nm in a spectrophotometer.

Measurement of Lipid peroxidation

MDA content was measured according to the procedure of Ohkawa et al. using 1,1,3,3-tetramethoxypropane as standard [23]. Briefly, to 100 μL of homogenate, 200 μL of 8.1 % SDS, and 1.5 mL of 20 % acetic acid, 1.5 mL of 0.8 % aqueous TBA solution was added, and the solution was made up to 4 mL. The solution was heated on boiling water bath for 60 min and then cooled, and 1 mL of distilled water was added. 5 mL butanol and pyridine (15:1) was added, and the mixture was shaken well. The mixture was then centrifuged at 4000 rpm for 10 min. The absorbance of orange layer was read at 532 nm.

Determination of total (T-SH), protein (P-SH), and nonprotein thiol (NP-SH) levels

The thiol groups were determined according to the procedure of Sedlak and Lindsay [24]. For T-SH, briefly, aliquots of 250 μL of the tissue homogenate were mixed in 5-mL test tubes with 750 μL of 0.2 M Tris buffer, pH 8.2, and 50 μL of 0.01 M 5, 5′-dithiobis (2-nitrobenzoic acid (DTNB)). The mixture was made up to 5 mL with 3950 μL of absolute methanol. A reagent blank and a sample blank were prepared in a same manner. The test tubes were stoppered with rubber caps. Color developed in 15 min, and the reaction mixture was centrifuged approximately at 3000×*g* at room temperature for 15 min. The absorbance of the supernatants was read in a spectrophotometer at 412 nm. Molar extinction coefficient at 412 nm was 13,100 M^{-1} cm^{-1} in both T-SH and NP-SH procedures.

For NP-SH, aliquots of 250 μL of the homogenates were mixed in 5-mL test tubes with 200 μL dis. water and 50 μL of 50 % TCA. The test tubes were shaken intermittently for 10 min and centrifuged for 15 min at approximately 3000×*g*. 200 μL of the supernatant was mixed with 400 μL of 0.4 M Tris buffer, pH 8.9; 10 μL of DTNB was

added; and the sample was shaken on a shaker. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank without homogenate. The protein thiols groups were calculated by subtracting the nonprotein thiol from total thiols.

Protein measurement

Total protein content of tissue sample was measured by the method of Lowry et al. using BSA as a standard [25].

Statistical Analysis

All the data were expressed as mean ± SE and were analyzed within a two-factor analysis of variance (ANOVA) between groups and regions. When a significant F ratio was found, Duncan's multiple range tests (DMRT) were used to assess the differences between group means. The statistical analysis was performed using SPSS 20 software package for windows. Probability values $p < 0.05$ were considered significant.

Results

Body weight changes

Table 1 represents the body weight changes in the swim-trained and *Dh* extract-supplemented groups. In the 4-month-old animals, the body weights were significantly decreased in the supplemented trainees compared to SE-C(N). However, in 18-month-old animals, both the supplemented trainees and sedentaries showed reduction in body weight over the SE-C(N).

Antioxidant enzymes

The SOD activity is significantly increased in all the groups of 4-month-old animals as represented in Table 2. Highest activity was evident in SE-C(+Dh_2) by 53, 70, and 67 % over the SE-C(N) in the CC, HC, and CB, respectively. In 18-month-old animals, region-specific changes were noticeable between CC and HC (Table 3). The activity was significantly enhanced in CC (42 %), HC (37 %), and CB (22 %) of SW-T(+Dh_2) with respect to SE-C(N).

CAT activity showed a similar trend as that of SOD, wherein the activity in 4-month-old animals was significantly enhanced in all the experimental groups (Table 2). The SE-C(+Dh_2) group exhibited higher activity by 56, 36, and 35 % compared to SE-C(N) in the CC, HC, and CB, respectively. However, in 18-month-old animals, activity was increased in both SW-T(+Dh_2) and SE-C(+Dh_2) over the unsupplemented sedentaries. (Table 3). Regional significance was evident in both the age groups.

A remarkable aspect of the GPx activity was that insignificant changes were noticed between the regions. In 4-month-old animals, SE-C(+Dh_2) group showed higher activity by 28, 15, and 35 % over the SE-C(N) in CC, HC, and CB, respectively (Table 2). However, in 18-month-old animals, significant increases of 58, 32, and 25 % were evident in CC, HC, and CB of SW-T(+Dh_2) animals (Table 3).

Markers of oxidative stress

SOR levels were measured in the brain as it is one of the major free radicals produced as a consequence of the physiological metabolic reactions in the central nervous system. SOR increased with age in all the regions with HC

Table 1 Body weight changes in the 4- and 18-month-old animals during the training period

Age (in months)	Groups	1st week	2nd week	3rd week	4th week
4	SE-C(N)	230 ± 9.12	233 ± 13.1	243 ± 13.1*	254 ± 9.10*
	SW-T(N)	230 ± 10.8	240 ± 14.7	245 ± 12.6	253 ± 16.5
	SW-T(+Dh_1)	213 ± 7.50	210 ± 12.9	237 ± 8.80	237 ± 8.80#
	SW-T(+Dh_2)	215 ± 2.90	223 ± 3.30	223 ± 3.30	230 ± 5.80#
	SE-C(+Dh_1)	228 ± 4.80	240 ± 7.10	255 ± 8.70	258 ± 8.50
	SE-C(+Dh_2)	227 ± 6.70	237 ± 12.0	243 ± 14.5	253 ± 14.5#
18	SE-C(N)	459 ± 0.57	464 ± 0.88*	469 ± 0.57*	475 ± 0.57*
	SW-T(N)	457 ± 1.45	462 ± 1.14	466 ± 0.88	470 ± 1.15#
	SW-T(+Dh_1)	455 ± 2.08	461 ± 3.60	464 ± 1.00	468 ± 1.52#
	SW-T(+Dh_2)	454 ± 1.45	456 ± 1.15	458 ± 0.88	461 ± 1.15#
	SE-C(+Dh_1)	456 ± 1.15	459 ± 2.64	464 ± 0.58	472 ± 3.28#
	SE-C(+Dh_2)	457 ± 0.88	462 ± 0.88	465 ± 0.88	469 ± 0.66#

* Significance in comparison with the 1st week

Significance between the sedentary control and experimental groups

*,# $p < 0.05$ was considered significant. Values are mean ± SE of five animals/group

Table 2 Antioxidant enzyme activities in discrete brain regions of the 4-month-old rats

Parameters	Groups					
4 months	SE-C(N)	SW-T(N)	SW-T(+Dh_1)	SW-T(+Dh_2)	SE-C(+Dh_1)	SE-C(+Dh_2)
SOD						
		*	*	*	*	*
CC	11.25 ± 0.3	14.73 ± 0.3	15.54 ± 0.3	17.34 ± 0.7	24.01 ± 0.4	23.25 ± 0.4
HC	8.63 ± 0.1	11.61 ± 1.8	13.71 ± 0.1	18.84 ± 0.2	28.96 ± 1.7	22.26 ± 0.5
CB	8.75 ± 0.1	11.27 ± 1.7	14.89 ± 0.3	15.16 ± 0.4	27.57 ± 0.6	24.42 ± 2.0
CAT						
		*	*	*	*	*
CC	22.30 ± 2.0	36.09 ± 1.5	50.92 ± 0.2	39.16 ± 1.3	69.65 ± 1.1	61.23 ± 1.9
HC	30.61 ± 1.4	34.77 ± 1.7	47.72 ± 0.2	46.01 ± 0.3	68.89 ± 3.0	65.22 ± 0.2
CB	29.63 ± 2.1	37.98 ± 0.4	45.28 ± 2.2	36.56 ± 1.9	65.16 ± 2.9	63.04 ± 0.5#
GPx						
		*		*	*	*
CC	17.45 ± 0.1	16.13 ± 1.5	18.66 ± 1.3	17.42 ± 0.6	24.25 ± 1.0	20.92 ± 2.0
HC	17.74 ± 1.3	13.15 ± 2.9	17.95 ± 0.4	17.77 ± 0.9	20.97 ± 3.0	18.96 ± 1.0
CB	16.07 ± 1.1	18.00 ± 0.5	17.11 ± 0.2	19.06 ± 3.0	24.66 ± 2.6	18.94 ± 0.7

* Significance between the sedentary control and experimental groups

Comparison between the regions

*,# $p < 0.05$ was considered significant. Data are expressed as mean ± SE of five animals/group and analyzed through two-way ANOVA followed by Duncan's multiple range tests. SOD activity is expressed as Units/mg protein; CAT as μmoles/mg protein; and GPx as nmoles of NADPH oxidized/min/mg protein

Table 3 Antioxidant enzyme activities in discrete brain regions of the 18-month-old rats

Parameter	Groups					
18 months	SE-C(N)	SW-T(N)	SW-T(+Dh_1)	SW-T(+Dh_2)	SE-C(+Dh_1)	SE-C(+Dh_2)
SOD						
		*	*	*	*	*
CC	12.57 ± 0.3	16.42 ± 2.5	16.72 ± 3.2	17.34 ± 1.7	21.04 ± 1.8	21.78 ± 2.1
HC	15.25 ± 1.6	15.65 ± 2.2	20.39 ± 1.5	18.84 ± 2.0	22.64 ± 2.6	24.11 ± 2.4#
CB	17.24 ± 0.7	17.87 ± 3.0	21.71 ± 2.1	15.16 ± 1.2	26.31 ± 1.9	22.09 ± 2.0#
CAT						
		*	*	*	*	*
CC	16.96 ± 0.8	24.54 ± 1.1	30.64 ± 1.2	39.29 ± 1.4	40.69 ± 1.5	40.49 ± 1.6
HC	19.39 ± 1.0	21.67 ± 1.7	37.48 ± 1.3	42.83 ± 1.6	39.67 ± 1.3	43.04 ± 1.0#
CB	18.25 ± 1.2	21.47 ± 1.6	37.84 ± 1.0	35.38 ± 0.8	44.56 ± 0.8	44.69 ± 1.2#
GPx						
		*	*	*	*	*
CC	12.32 ± 0.9	17.44 ± 0.6	17.50 ± 1.4	23.56 ± 1.6	25.19 ± 1.2	29.53 ± 1.6
HC	14.31 ± 1.1	18.48 ± 0.9	22.05 ± 1.0	20.09 ± 0.9	21.81 ± 1.1	21.25 ± 1.1
CB	17.25 ± 1.2	17.39 ± 1.2	20.90 ± 1.5	20.27 ± 0.8	22.77 ± 1.3	22.98 ± 1.2

* Significance between the sedentary control and experimental groups

Comparison between the regions

*,# $p < 0.05$ was considered significant. Data are expressed as mean ± SE of five animals/group and analyzed through two-way ANOVA followed by Duncan's multiple range tests. SOD activity is expressed as Units/mg protein; CAT as μmoles/mg protein; and GPx as nmoles of NADPH oxidized/min/mg protein. Five animals/group

showing the highest concentration in 4- and 18-month-old (Fig. 1a, b) animals. Exercise as well as *Dh* extract (Dh_2) per se was effective in reducing the superoxide level in the CC (29 %), HC (46 %), and CB (23 %) of 4-month-old animals. A noticeable feature in the 18-month-old animals was an insignificant change in the superoxide level

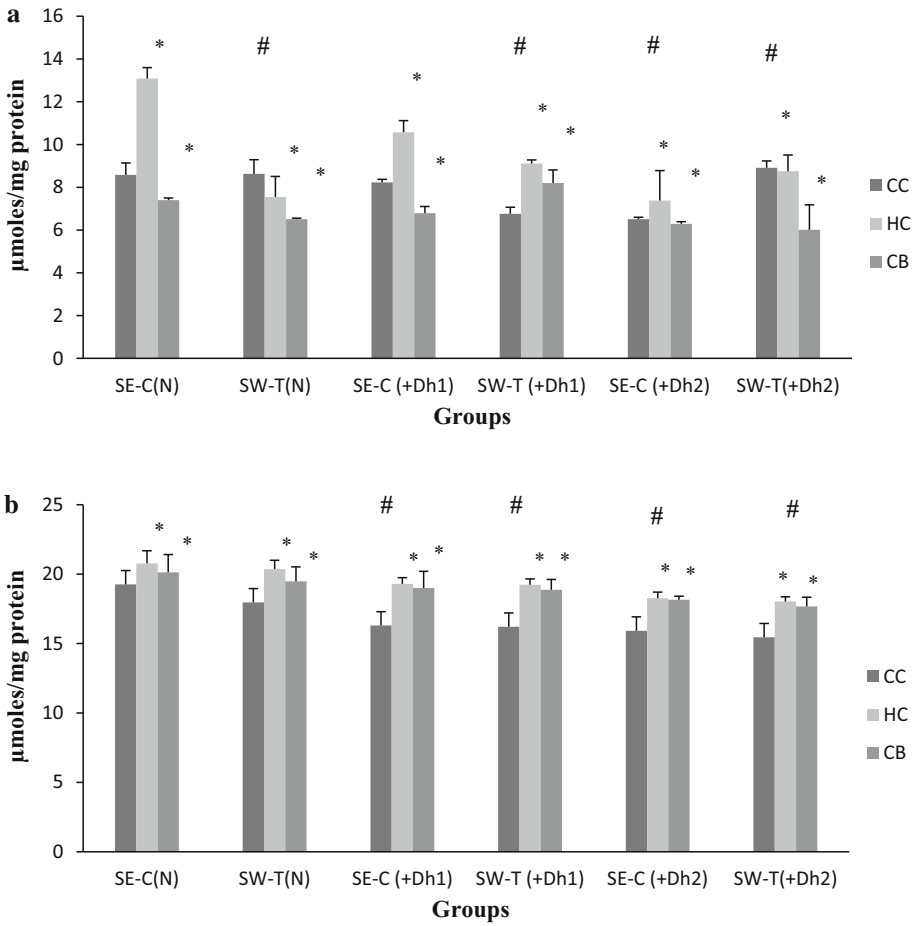

Fig. 1 The levels of superoxide radical in discrete brain regions of **a** 4- and **b** 18-month-old animals of control and experimental groups. Values are mean ± SE of five animals/group. Significance between group means was analyzed by Tukey's test and statistical significance set at $p < 0.05$. *Asterisks* depict the comparison of hippocampus (HC) and cerebellum (CB) with cerebral cortex (CC). *Hashes* depict the comparison of experimental groups with controls. SE-C(N), normal sedentary controls; SE-C(+Dh_1) and SE-C(+Dh_2) sedentary supplemented groups with 50 and 100 mg of *Dh* extract/kg b.w.; SW-T(N), normal swim trainees; SW-T(+Dh_1) and SW-T(+Dh_2), swim trainees supplemented with 50 and 100 mg of *Dh* extract/kg b.w

between supplemented trainees and sedentaries when compared to SE-C(N).

The protein carbonyl (PC) content, marker of protein oxidation, was found to be elevated with age with HC showing the higher content than the CC in both the age groups. The carbonyl content was decreased maximally in SW-T(+Dh_2) in all the three regions of 4 (CC-23 %, HC-30 %, CB-16 %)- and 18-month-old animals (CC-53 %, HC-46 %, CB-70 %) over the SE-C(N), respectively (Fig. 2a, b).

The MDA content was assessed in different regions of the brain as a marker of lipid peroxidation. Age-related increase in the MDA content was evident with CC showing the highest content in 4- and 18-month-old animals. In the 4-month-old animals, lower MDA content was noticed in all the experimental groups. However, the changes were insignificant between supplemented trainees and sedentaries (Fig. 3a). However, the 18-month-old animals showed reduced MDA content in the SW-T(+Dh_2) by 46 % (CC) and 38 % (HC and CB) over their SE-C(N) (Fig. 3b).

Thiols

Age-related changes in T-SH, NP-SH, and P-SH levels of 4- and 18-month-old animals are represented in Tables 4 and 5. In 4-month-old animals, T-SH levels were found to be significantly increased in all the experimental groups. Training and supplementation of *Dh* extract (Dh_2) were more effective in elevating the T-SH levels by 21 % (CC), 17 % (HC), and 18 % (CB) over the SE-C(N). However, in 18-month-old animals, significant increases of 18 % (CC), 13 % (HC), and 15 % (CB) were evident in SW-T(+Dh_2) animals in relation to their SE-C(N). Region-specific changes were noticeable in 4- and 18-month-old animals.

Age-related decrease in NP-SH levels were observed with HC showing the highest content compared to other regions. The levels were also found to be maximally enhanced in SW-T(+Dh_2) with respect to SE-C(N) by 18 % (CC), 13 % (HC), and 15 % (CB) in 4-month-old animals. However, in 18-month-old animals, NP-SH levels

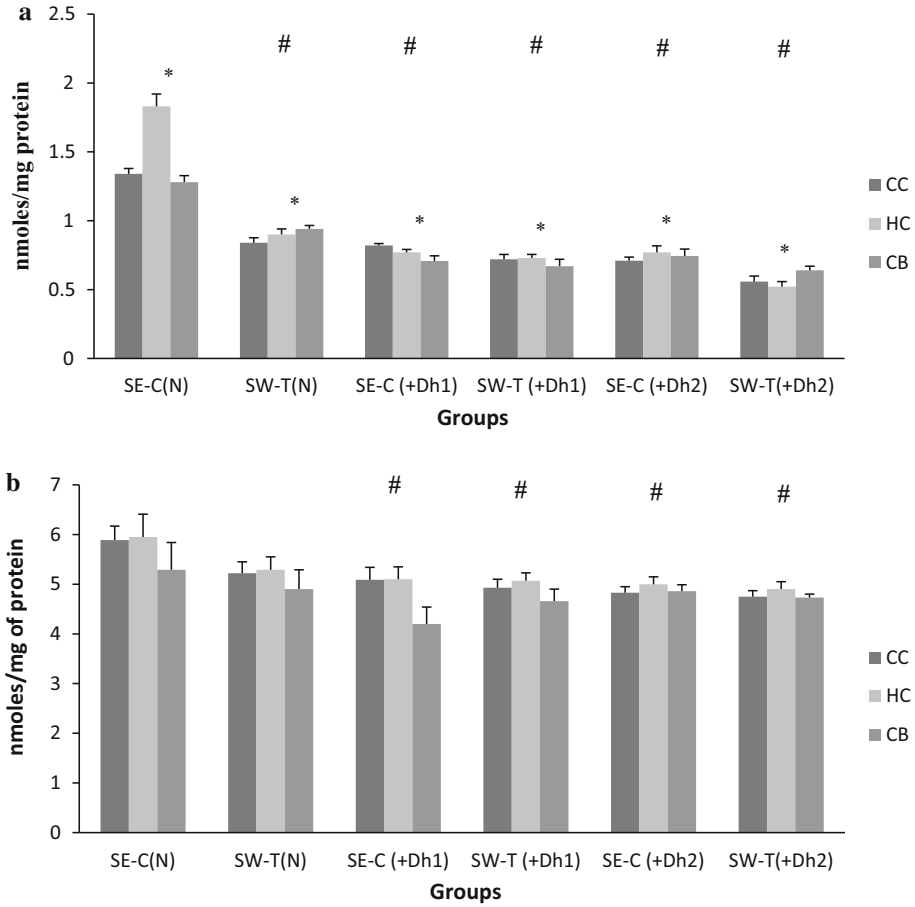

Fig. 2 The levels of protein carbonyl in discrete brain regions of **a** 4- and **b** 18-month-old animals of control and experimental groups. Values are mean ± SE of five animals/group. Significance between group means was analyzed by Tukey's test and statistical significance set at $p < 0.05$. *Asterisks* depict the comparison of hippocampus (HC) and cerebellum (CB) with cerebral cortex (CC). *Hashes* depict the comparison of experimental groups with controls. SE-C(N), normal sedentary controls; SE-C(+Dh_1) and SE-C(+Dh_2) sedentary supplemented groups with 50 and 100 mg of *Dh* extract/kg b.w.; SW-T(N), normal swim trainees; SW-T(+Dh_1) and SW-T(+Dh_2), swim trainees supplemented with 50 and 100 mg of *Dh* extract/kg b.w

were found to be higher in both Dh_2-supplemented sedentaries and trainees.

The P-SH levels were found be decreased with age. In 4-month-old animals, region-specific changes were evident and the P-SH levels were found to be higher in both SE-C(+Dh_2) and SW-T(+Dh_2) over unsupplemented sedentaries. A notable feature in 18-month-old animals is that insignificant changes were noticed between the regions as well as between the supplemented sedentaries and trainees.

Discussion

There has been a major focus on antioxidant properties of nutraceuticals and their influence on the antioxidant status of brain. Epidemiological studies show a link between plant-derived compounds and health benefits. The roots of *D. hamiltonii* are consumed for various health benefits. Studies have shown that the aqueous root extract has protective effect against neurotoxicity and Parkinson's disease [26, 27]. Along with the consumption of natural products, physical exercise also improves the antioxidant status of the brain [28]. Therefore, the purpose of the present study was to determine whether the combined effects of *Dh* supplementation and exercise have a better protective effect in attenuating age-related oxidative stress in the brain. We chose CC, HC, and CB in our study as they are critical regions involved in the higher brain functions and vary in cellular as well as regional distribution of antioxidant biochemical defenses [29].

We chose swimming as physical exercise as these animals are inborn swimmers and are less stressed when compared to treadmill exercise [30], and also there is no requirement of electric shock to promote this exercise protocol. In the present study, the body weight is decreased in swim-trained and supplemented groups, which indicates the beneficial effects of exercise training and supplementation of root extract. Earlier, we have reported reduction in the body weight of rats subjected to swimming training at 3 % intensity for 30 days [31]. Studies by Bais et al. and Boque et al. reported that supplementation with plant extract polyphenols decreased body weight gain in obese rats [32, 33].

SOD is the major enzyme involved in the dismutation of superoxide radical into less toxic hydrogen peroxide, which is further detoxified into water and oxygen by CAT and

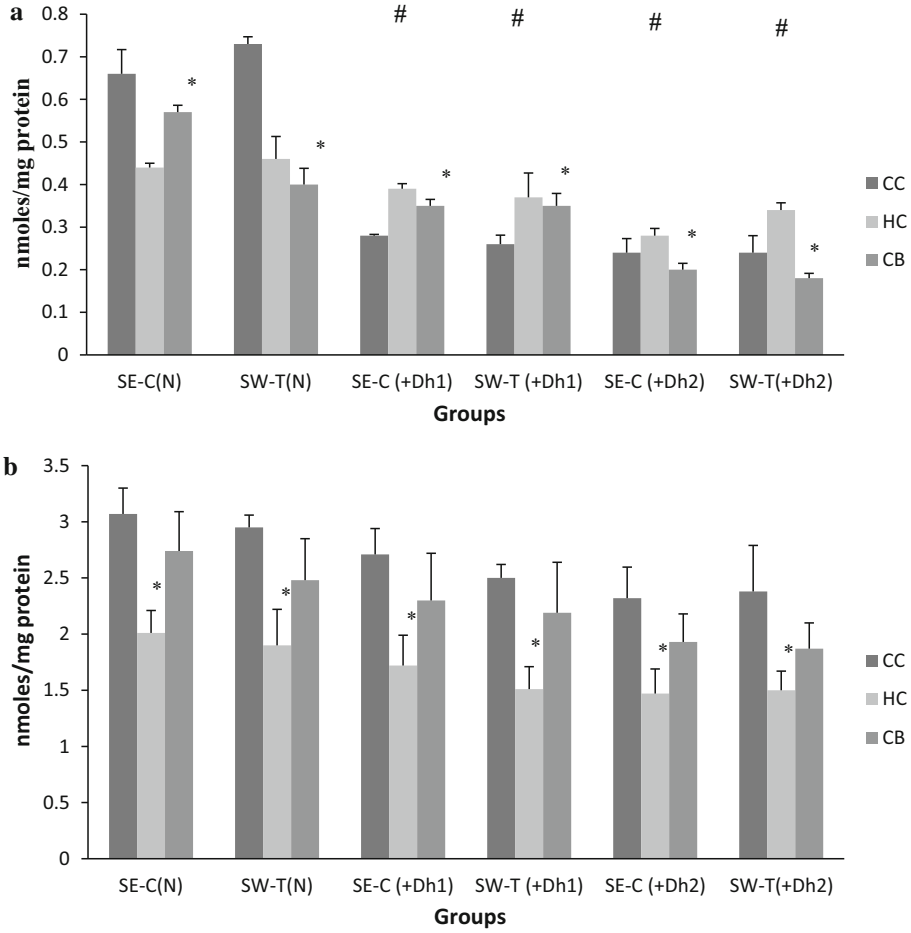

Fig. 3 The levels of MDA in discrete brain regions of **a** 4- and **b** 18-month-old animals of control and experimental groups. Values are mean ± SE of five animals/group. Significance between group means was analyzed by Tukey's test and statistical significance set at $p < 0.05$. *Asterisks* depict the comparison of hippocampus (HC) and cerebellum (CB) with cerebral cortex (CC). *Hashes* depict the comparison of experimental groups with controls. SE-C(N), normal sedentary controls; SE-C(+Dh_1) and SE-C(+Dh_2) sedentary supplemented groups with 50 and 100 mg of *Dh* extract/kg b.w.; SW-T(N), normal swim trainees; SW-T(+Dh_1) and SW-T(+Dh_2), swim trainees supplemented with 50 and 100 mg of *Dh* extract/kg b.w

Table 4 The levels of T-SH, NP-SH, and P-SH in discrete brain regions of control and experimental groups in 4-month-old rats

Parameters	Groups					
4 months	SE-C(N)	SW-T(N)	SW-T(+Dh_1)	SW-T(+Dh_2)	SE-C(+Dh_1)	SE-C(+Dh_2)
T-SH						
		*	*	*	*	*
CC	118.65 ± 4.8	127.7 ± 2.4	135.8 ± 3.1	149.6 ± 3.3	130.36 ± 2.8	145.1 ± 3.4
HC	134.32 ± 1.9	138.4 ± 1.4	144.7 ± 3.4	162.5 ± 5.6	141.14 ± 2.9	152.8 ± 2.3#
CB	127.97 ± 1.8	131.8 ± 1.7	140.6 ± 4.2	155.2 ± 7.9	136.62 ± 3.7	148.5 ± 4.4#
NP-SH						
			*	*	*	*
CC	78.83 ± 2.00	81.94 ± 2.2	85.44 ± 4.2	96.51 ± 4.6	82.31 ± 3.93	91.31 ± 2.5
HC	88.23 ± 2.20	89.97 ± 2.7	93.54 ± 4.1	101.9 ± 6.3	92.35 ± 3.90	95.42 ± 3.5#
CB	85.60 ± 2.80	86.00 ± 4.2	89.92 ± 7.3	100.3 ± 6.0	86.40 ± 1.95	92.20 ± 4.6#
P-SH						
		*	*	*	*	*
CC	39.82 ± 2.57	45.77 ± 4.5	50.36 ± 7.3	54.61 ± 1.3	48.06 ± 1.10	53.78 ± 3.6
HC	46.09 ± 3.85	48.39 ± 2.3	51.23 ± 7.5	60.63 ± 1.7	51.01 ± 6.83	57.39 ± 1.4#
CB	42.34 ± 1.90	45.81 ± 2.5	50.65 ± 3.1	59.98 ± 1.9	50.21 ± 1.76	56.29 ± 3.6

* Significance between the sedentary control and experimental groups

Comparison between the regions

*,# $p < 0.05$ was considered significant. Data are expressed as mean ± SE of five animals/group and analyzed through two-way ANOVA followed by Duncan's multiple range tests. Units: nmol/mg protein

Table 5 The levels of T-SH, NP-SH, and P-SH in discrete brain regions of control and experimental groups in 18-month-old rats

Parameters	Groups					
18 months	SE-C(N)	SW-T(N)	SW-T(+Dh_1)	SW-T(+Dh_2)	SE-C(+Dh_1)	SE-C(+Dh_2)
T-SH						
			*	*	*	*
CC	94.14 ± 5.40	99.34 ± 5.17	110.2 ± 4.54	112.8 ± 5.69	104.9 ± 5.12	112.58 ± 4.55
HC	93.22 ± 3.77	98.87 ± 2.63	120.2 ± 3.94	129.5 ± 3.11	119.4 ± 3.20	123.54 ± 2.56#
CB	102.0 ± 2.36	103.6 ± 3.01	108.5 ± 3.64	110.9 ± 4.67	105.3 ± 31.0	110.32 ± 2.63#
NP-SH						
					*	*
CC	54.10 ± 4.62	55.9 ± 3.77	60.70 ± 2.37	62.08 ± 1.87	56.70 ± 3.01	61.57 ± 2.71
HC	56.78 ± 2.42	57.1 ± 3.01	65.50 ± 2.42	72.25 ± 2.23	65.29 ± 1.47	68.10 ± 1.66#
CB	54.27 ± 2.48	54.7 ± 2.30	58.20 ± 2.30	59.53 ± 1.15	55.65 ± 1.99	59.17 ± 2.30
P-SH						
				*	*	*
CC	40.16 ± 2.88	43.5 ± 1.51	49.50 ± 1.28	50.76 ± 0.91	48.17 ± 0.88	51.01 ± 1.28
HC	36.44 ± 2.10	41.0 ± 1.90	54.68 ± 1.21	57.25 ± 1.73	54.13 ± 1.44	55.44 ± 1.78
CB	47.74 ± 1.44	48.9 ± 1.96	50.26 ± 0.71	51.4 ± 2.48	49.65 ± 1.28	51.15 ± 2.10

* Significance between the sedentary control and experimental groups

Comparison between the regions

*,# $p < 0.05$ was considered significant. Data are expressed as mean ± SE of five animals/group and analyzed through two-way ANOVA followed by Duncan's multiple range tests. Units: nmol/mg protein

GPx. Our study demonstrated increased SOD activity in the CC, HC, and CB regions of all the experimental groups. The enhanced activity indicates the synergistic effects of exercise and *Dh* extract to scavenge the SOR in the brain. A similar trend is noticed in rat brain regions subjected to swimming and treadmill training [10, 34]. The increased activity in the supplemented animals may be due to *Dh* extract, which is very rich in polyphenols and could be a source of dietary antioxidants in neuroprotection in enhancing the antioxidant status of brain [13].

CAT and GPx activity was found to be decreased with age. The decrease activity may be attributed to age-related response to oxidative stress. The higher activities were evident in SE-C(+Dh_2) and SW-T(+Dh_2) groups. The GPx activity did not show any significant changes between regions when compared to CAT, suggesting that activities of these enzymes may vary depending on type, duration, intensity of exercise, and dosage of supplementation. Gomez-Pinilla and Ying revealed that exercise and diet help in maintaining brain plasticity and health in HC and hypothalamus in rats [35]. Studies by Balu et al. demonstrated that polyphenols from grape seed extract helps in diminishing oxidative stress in the central nervous system of aged rats [4].

The exact mechanism of action by which the Dh extract upregulates the antioxidant enzyme activities is not clearly known. It may be due to the increased expression of the corresponding genes by interaction with the antioxidant response elements (ARE) that transcriptionally regulate these genes. Further investigation is required regarding the interactions of antioxidant compounds in the extract with the AREs in vivo [36, 37]. Previous studies have shown that the supplementation of Dh extract enhances the antioxidant enzyme activities in stomach, liver, and brain [13, 38, 39].

SOR, one of the major free radicals, was found to be higher in the HC compared to other regions. The superoxide levels were significantly reduced in supplemented swim trainees, which indicates the synergistic effects of exercise and supplementation. Aksu et al. showed that chronic exercise has a favorable effect on HC, possibly by decreasing SOR formation [40]. The reduced level of SOR is an evidence for the increased activity of SOD enzymes as these are involved in dismutation of these radicals.

PCs have been used as a measure to estimate protein damage/oxidation in cells and tissues [41]. Protein oxidation, an exothermic event where peptides react with free radicals, causes the modification of several amino acids, protein aggregation, and protein fragmentation. In the present study, PC content was decreased maximally in SW-T(+Dh_2) group of 4- and 18-month-old animals. HC exhibited a higher carbonyl content compared to other regions, since this area is particularly vulnerable to oxidative stress [4]. Increased protein oxidation in HC is

also documented in Alzheimer's patients [42]. Our results suggest that regular exercise with supplementation of *Dh* extract decreases the accumulation of oxidative modification of proteins. Radak et al. reported that regular exercise improves cognitive function in parallel with a decrease in the accumulation of oxidative modification of proteins [43]. Studies by Jolitha et al. found that moderate swimming exercise training complimented with vitamin E reduces the risk of oxidative modification of proteins and lipids and can substantially stimulate the endogenous antioxidant system [16].

MDA is one of the advanced lipid peroxidation products. Our results indicate that the swimming training, supplementation, and their combined effects are beneficial in curtailing lipid peroxidation in the brain regions of both the age groups. Studies by Skryzdlewska et al. showed that the polyphenols from green tea helps in antioxidative capacity of the brain tissue and in lowering the level of lipid peroxidation products [44]. Devi and Kiran reported that the integration of vitamin E and exercise may act as useful neuroprotector against age-related decline in the antioxidant enzymes and increased lipid peroxidation [10].

Thiols constitute the major portion of the total body antioxidants, and they play a significant role in defense against ROS. The present study demonstrated that swim training and supplementation significantly increased the thiol levels in aged rats. There is an age-dependent reduction in thiol concentration in the brain regions, which indicates that the efficiency of S-thiolation as a mechanism of defense that decreases with age, which creates an increased risk of irreversible oxidation of sulfhydryl (–SH) groups of proteins [45]. The reduced levels of thiol implies depleted antioxidant capacity of the brain, disturbance in the balance between different redox forms of thiols which in turn lead to impaired protection of P-SH groups upon irreversible oxidation.

In conclusion, our results suggest that supplementation of *Dh* extract has a potential to enhance the enzymatic antioxidant defense. Regular exercise upregulates the antioxidant defense by reducing the level of SOR, protein oxidation, and lipid peroxidation. The adaptive effect of these two treatments was more profound in enhancing the antioxidant status. The present study revealed that nutrition and physical exercise play an important role in maintaining brain health.

Acknowledgments This work was supported by Bangalore University Internal Research Fund (Grant No. DEV/D2/YRB/BUIRF/2010-11).

Compliance with ethical standards

Conflict of interests The authors declare that there are no conflicts of interest.

References

1. Flores MF, Martins A, Schimidt LH, Santos FW, Izquiredo I, Mello-Carpes PB, Carpes PF (2014) Effects of green tea and physical exercise on memory impairments associated with aging. NeurochemInt 78:53–60
2. Cui H, Kong Y, Zhang H (2012) Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 2012:1–13
3. Butterfield DA, Stadtman ER (1997) Protein oxidation processes in aging brain. Adv Cell Aging Gerontol 2:161–191
4. 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. Int J Dev Neurosci 23:501–507
5. Asl NA, Sheikhzade F, Torchi M, Roshangar L, Khamnei S (2008) Long-term regular exercise promotes memory and learning in young but not in older rats. Pathophysiology 15:9–12
6. Suominen-Troyer S, Davis KJ, Ismail AH, Salvendy G (1986) Impact of physical fitness on strategy development in decision making tasks. Percept Mot Skills 62:71–77
7. Kramer AF, Hahn S, Cohen NJ, Banich MT, McAuley E, Harrison CR, Chason J, Vakil E, Bardell L, Bioleau RA, Colcombe A (1999) Ageing, fitness and neurocognitive function. Nature 400:418–419
8. Fordyce DE, Farrar RP (1991) Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. Behav Brain Res 46:123–133
9. VanPraag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning and long-term potentiation in mice. Proc Natl Acad Sci 96:13427–13431
10. Devi SA, Kiran TR (2004) Regional responses in antioxidant system to exercise training and dietary Vitamin E in aging rat brain. Neurobiol Aging 25:501–508
11. Radak Z, Koltai E, Taylor AW, Higuchi M, Kumagai S, Goto S, Boldogh I (2013) Redox-regulating sirtuins in aging, caloric restriction, and exercise. Free Radic Biol Med 58:87–97
12. Wiswedel I, Hirsch D, Kropf S, Gruening M, Pfister E, Schewe T, Sies H (2004) Flavonol-rich cocoa drink lowers plasma F2-isoprostane concentrations in humans. Free Radic Biol Med 37:411–421
13. Srivastava A, Shivanandappa T (2010) Neuroprotective effect of *Decalepis hamiltonii* roots against ethanol-induced oxidative stress. Food Chem 119:626–629
14. Srivastava A, Harish SR, Shivanandappa T (2006) Antioxidant activity of the roots of Decalepis hamiltonii (Wight and Arn.). LWT 39:1059–1065
15. Naveen S, Khanum F (2010) Antidiabetic, antiatheroscerotic and hepatoprotective properties of *Decalepis hamiltonii* in streptozotocin-induced diabetic rats. J Food Biochem 34:1231–1248
16. Jolitha AB, Subramanyam MVV, Devi SA (2006) Modification by vitamin E and exercise of oxidative stress in regions of aging rat brain: studies on superoxide dismutase isoenzymes and protein oxidation status. Exp Gerontol 41:753–763
17. Todly A, Stadler K, Sasvari M, Jakus J, Jung KJ, Chung H, Berkes I, Nyakas C, Radak Z (2005) The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res Bull 65:487–493
18. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
19. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:140–147
20. Flohe L, Gunzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–121

21. Das UN, Padma M, Sagar PS, Ramesh G, Koratkar R (1990) Stimulation of free radical generation in human leukocytes by various agents including tumour necrosis factor is a calmodulin dependent process. Biochem Biophys Res Commun 167:1030–1036
22. Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357
23. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anals Biochem 95:351–358
24. Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and non protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
26. Zarei M, Shivanandappa T (2013) Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of *Decalepis hamiltonii* in mice. Food Chem Toxicol 57:179–184
27. Jahromi SR, Haddadi M, Shivanandappa T, Ramesh SR (2015) Attenuation of neuromotor deficits by natural antioxidants of *Decalepis hamiltonii* in transgenic Drosophila model of Parkinson's disease. Neuroscience 293:136–150
28. Anand S, Rajashekharaiah V, Tekupalli R (2015) Effect of age and physical activity on oxidative stress parameters in experimental rat model. Int J Clin Exp Physiol 2:185–190
29. Verma RS, Srivastava N (2001) Chlorpyrifos induced alterations in levels of thiobarbituric acid reactive substances and glutathione in rat brain. Ind J Exp Biol 39:174–177
30. Devi SA, Prathima S, Subramanyam MVV (2003) Dietary vitamin E and physical exercise: II. Antioxidant status and lipofuscin-like substances in aging rat heart. Exp Gerontol 38:291–297
31. Anand S, Devi SA, Ravikiran T (2014) Differential expression of the cerebral cortex proteome in physically trained adult rats. Brain Res Bull 104:88–91
32. Bais S, Singh GS, Sharma R (2014) Antiobesity and hypolipidemic activity of *Moring oleifera* leaves against high fat diet-induced obesity in rats. Adv Bio 2014:1–9
33. Boque N, Campion J, Iglesia R, Garza AL, Milagro FI, Roman BS, Banuelos O, Martinez JA (2013) Screening of polyphenolic plant extracts for anti-obesity properties in Wistar rats. J Sci Food Agric 93:1226–1232
34. Rybak LP, Somani SM, Ravi R (1995) Effect of exercise training on antioxidant system in brain regions of rat. Pharmacol Biochem Behav 50:635–639
35. Gomez-Pinilla F, Ying Z (2010) Differential effects of exercise and dietary docosahexaenoic acid (DHA) on molecular systems associated with control of allostasis in the hypothalamus and hippocampus. Neuroscience 168:130–137
36. Mandel SA, Amit T, Kalfon L, Reznichenko L, Weinreb O, Youdim MB (2008) Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). J Alzheimers Dis 15:211–222
37. Moskaug JO, Carlsen H, Myhrstad MC, Blomhoff R (2005) Polyphenols and glutathione synthesis regulation. Am J Clin Nutr 81:277S–283S
38. Srikanta BM, Siddaraju MN, Dharmesh SM (2007) A novel phenol-bound pectic polysaccharide from *Decalepis hamiltonii* with multi-step ulcer preventive activity. World J Gastroenterol 13(39):5196–5207
39. Srivastava A, Shivanandappa T (2010) Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. Food Chem 118:411–417
40. Aksu I, Topcu A, Camsari UM, Acikgoz O (2009) Effect of acute and chronic exercise on oxidant-antioxidant equilibrium in rat hippocampus, prefrontal cortex and striatum. Neurosci Lett 452:281–285
41. Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 32:790–796
42. Hensely K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, Lovell M, Markesbery WR, Butterfield DA (1995) Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem 65:2146–2156
43. Radak Z, Kanek T, Tahara S, Nakamoto H, Pucsok J, Sasvari M, Nyakas C, Goto S (2001) Regular exercise improves cognitive function and decreases oxidative damage in rat brain. Neurochem Int 38:17–23
44. Skryzdlewska E, Ostrowska J, Stankiewicz A, Fabisszewski R (2002) Green tea as a potent antioxidant in alcohol intoxication. Addict Biol 7:307–314
45. MałgorzataI Grazyna C, Elzbieta L-K, Edward B, Lidia W (2004) Plasma levels of total, free and protein bound thiols as well as sulfane sulfur in different age groups of rats. Acta Biochem Polonica 51:815–824