

Emerging role of *Centella asiatica* in improving age-related neurological antioxidant status

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Abstract

Free radicals have been hypothesized to play an important role in ageing process. There exists an imbalance between free radical production and antioxidant defense mechanism, which may lead to cell death during ageing. Our study was designed to determine whether extract of *Centella asiatica*, an antioxidant, when administered orally (300 mg/kg body weight/day) for 60 days would prevent age-related changes in antioxidant defense system, lipid peroxidation (LPO) and protein carbonyl (PCO) content in rat brain regions such as cortex, hypothalamus, striatum, cerebellum and hippocampus. Aged rats elicited a significant decline in the antioxidant status and increased the LPO and PCO as compared to control rats in all five regions studied. The increase in LPO and PCO contents were (64%, 34%) in cortex, (86%, 30%) in cerebellum, (51%, 47%) in striatum, (77%, 27%) in hypothalamus and (58%, 45%) in hippocampus, respectively, in aged rats as compared to young rats. Supplementation of *C. asiatica* was effective in reducing brain regional LPO and PCO levels and in increasing the antioxidant status. Thus, *C. asiatica* by acting as a potent antioxidant exerted significant neuroprotective effect and proved efficacious in protecting rat brain against age related oxidative damage.

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1. Introduction

The ageing is a natural process, which represents various morphological and biochemical changes that occur from maturity to senescence, rendering the organism more vulnerable to disease and toxicity eventually leading to death. The survival of an organism depends on the ability to withstand the repeated challenges by a variety of environmental and endogenous toxins. As an organism ages, its ability to withstand these insults decreases (Bains and Shaw, 1997). According to the oxidative stress hypothesis of ageing, the senescence-associated loss of functional capacity is due to the accumulation of molecular oxidative damage (Sohal et al., 2002) by toxic free radicals produced during normal respiration. Oxidative damage may contribute to the ageing process and to the neuropathogenesis of several diseases including Stroke, Parkinson's

disease and Alzheimer's disease (Harman, 1993). Free radicals have previously been reported to be capable of damaging many cellular components such as proteins (Dean et al., 1997), lipids (Cai et al., 1996) and DNA (Hamilton et al., 2001) and cause glyco-oxidation (Saxena et al., 1999). Brain is particularly vulnerable to oxidative damage due to various factors like (a) high utilization of inspired oxygen, (b) high susceptibility of large amount of oxidizable polyunsaturated fatty acids that are prone to lipid peroxidation, (c) the abundance of redox-active transition metal ions, and (d) the relative dearth of antioxidant defense systems (Subbarao and Richardson, 1990; Ogawa, 1994)

To protect cells against oxidative damage by oxidants, produced during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organisms (Cebalás-picot et al., 1992). Major antioxidants like SOD, CAT, GSH-Px, glutathione, ascorbic acid, α tocopherol are important for cellular protection due to their ability to detoxify free radicals, such as reactive oxygen species (ROS) (Young and Woodside, 2001). Various synthetic antioxidants have been used, which restricted the use of natural antioxidants as in food, and were proven to be carcinogenic (Madavi and Salunkhe, 1995). Potential antioxidant therapy

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should, therefore, include either natural antioxidant or agents, which are capable of augmenting the function of these enzymes (Bast et al., 1991). Thus, a need for identifying alternative natural and safer sources of food antioxidant is created. Therefore, search for natural antioxidants, especially of plant origin, has notably increased in recent years (Loliger, 1991). And, none has attempted evaluating therapeutic intervention with the natural antioxidant like *Centella asiatica* in aged rat brain regions (cortex, hypothalamus, striatum, cerebellum and hippocampus).

Phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic effects (Youdim and Joseph, 2001). *C. asiatica* (L) urban, synonym *Hydrocotyle asiatica*, belongs to the family Apiaceae and is found almost all over the world. In Ayurveda, an Indian system of medicine, this is used in the management of central nervous system, skin and gastrointestinal disorder. The major principles in the plant are the polyphenols (Zainol et al., 2003) and triterpenes (Inamdar et al., 1996). *C. asiatica* has been shown to improve memory, general mental ability of mentally retarded children (Kuppurajan et al., 1978). It was also shown to have wound healing property (Suguna et al., 1996), anticancer property (Babu et al., 1995), antioxidant property (Zainol et al., 2003) and is also shown to have antileptrotic property (Sahu et al., 1989).

Therefore, our study was concentrated on the role of *C. asiatica* in augmenting the functions of antioxidants and decreasing the level of lipid peroxidation and protein carbonyls in aged rat brain regions.

2. Materials and methods

2.1. Plant material

Fresh whole plant of *C. asiatica* were collected from market and samples of the plant were identified and authenticated by botanist from Dr Sasikala Ethirajulu, Botanist, Dept of Pharmacognosy, Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS, New Delhi), Arumbakkam, Chennai, Tamil Nadu. The plants were cleaned, air-dried and were powdered. The powder was soaked in double distilled water, ethanol in shaking incubator at $25 \pm 1^\circ\text{C}$ (1:1) for 2 days. The extractive solution was filtered through Whatman No.1 filter paper and concentrated using concentrator. Then, it was frozen and subjected to lyophilization.

2.2. Source of chemicals

Bovine serum albumin was purchased from Sigma Chemical Company (St Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, CDH division, Mumbai, India and Sarabhai M. Chemicals, Baroda, India.

2.3. Animals

The laboratory animal protocol used for this study was approved by committee for the purpose of control and supervision on experimental animals (CPCSEA) at Dr ALM PG.IBMS, University of Madras, Chennai, India. Male albino rats of *Wistar* strain used in this study were obtained from King's Institute of Preventive Medicine, Chennai, and maintained in a clean rodent room. Animals were housed 2–3 per cage in cages that were fitted with stainless-steel wire-mesh bottoms and maintained at a temperature of $28 \pm 1^\circ\text{C}$, and under a daily photoperiod of 12-h light/dark cycle. The animals were fed with pellet diet (Hindustan Lever Limited, Mumbai, India) and tap water ad libitum. The commercial rat feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre (wt/wt) with adequate mineral and vitamin contents.

2.4. Grouping of animals

The animals were divided into four major groups, such as

- Group I: Young control rats (3–4-months-old weighing approximately 130–150 gm).
- Group II: Young rats + (*C. asiatica* extract treated for 60 days).
- Group III: Aged control rats (above 24-months-old weighing about 380–410 gm).
- Group IV: Aged rats + (*C. asiatica* extract treated for 60 days).

Each group consisted of six animals.

Extract of *C. asiatica* (300 mg/kg body weight/day) was dissolved in 0.89% physiological saline and administered orally. Control animals received physiological saline alone. On completion of experimental period, animals were killed by decapitation. Brain was excised immediately; regions were separated according to the method of Glowinski and Iversen (1966) and immersed in ice cold physiological saline. The tissue was homogenized using 0.01 M Tris-HCl (pH 7.4) and the supernatant was used for the assay of enzymatic and non-enzymatic antioxidant analysis.

For protein oxidation, the homogenates were prepared using 10 mM HEPES buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4 , and 0.6 mM MgSO_4 . The buffer also contained the following protease inhibitors to prevent proteolysis of oxidized proteins during homogenization: 0.5 $\mu\text{l/ml}$ leupeptin, 0.7 $\mu\text{g/ml}$ pepstatin, 0.5 $\mu\text{g/ml}$ aprotinin and 40 $\mu\text{g/ml}$ phenylmethylsulphonyl-fluoride. The homogenates were centrifuged at $40,000 \times g$ for 20 min and supernatant was used for the analysis of PCO.

To determine lipid peroxidation, homogenates were prepared in 0.01 M Tris buffer, pH 7.4, using a homogenizer and were used immediately.

2.4.1. Incubation

Homogenate aliquots (0.5 ml for lipid peroxidation and 0.5 ml for protein oxidation) in duplicate were incubated in a Dubnoff bath at 37 °C for 30 min (lipid peroxidation) or 60 min (protein oxidation) with no additions (spontaneous conditions) or after the addition of 5 µl of 1 µM freshly prepared aqueous solutions of FeCl₂ (stimulated conditions).

All the above steps (except incubation) were carried out at 4 °C.

2.5. Biochemical analysis and enzymes assays

2.5.1. Protein carbonyl content

Protein carbonyl contents in homogenates prepared from cortex, hypothalamus, striatum, cerebellum and hippocampus of each rat were analyzed using 2,4-dinitrophenylhydrazine (DNPH) method as described by Levine et al. (1990) with slight modifications. The homogenized tissue was transferred to a plastic tube, left for 15 min at room temperature, and then streptomycin sulfate solution (10 w/v%) was added to a final concentration of 1% to precipitate any extracted DNA which could react with DNPH and contribute to the carbonyl level. The solution was mixed and left to stand for further 15 min at room temperature, after which was centrifuged at 2800 g for 10 min at room temperature. Briefly, 300 µl of tissue supernatant containing 1.6–2.0 mg protein was pipetted into the tubes, to which 300 µl of 10 mM DNPH in 2 N HCl was added. HCL of 2N alone was taken as blank. Samples were then incubated for 1 h at room temperature, stirred every 10 min, precipitated with 10% TCA (final concentration) and centrifuged for 3 min at 16,000×g. The pellet was washed three times with 1ml ethanol/ethyl acetate (1:1, v/v) and redissolved in 1ml of 6 M guanidine in 10 mM phosphate buffer-trifluoroacetic acid (pH 2.3) and insoluble substance was removed by centrifugation. The difference in absorbance between the DNPH-treated and the HCL treated samples was determined at 366 nm and the results were expressed as nanomole carbonyl groups/mg protein using the extinction coefficient of 22.0 mM⁻¹ cm⁻¹ for aliphatic hydrazones.

2.5.2. Lipid peroxidation (LPO)

Lipid peroxides were estimated in the brain regions (the cortex, hypothalamus, striatum, cerebellum and hippocampus) using a modified thiobarbituric acid (TBA) test described by Ohkawa et al. (1979) using malondialdehyde (MDA) as the standard. Briefly, 0.1 ml of homogenate of brain regions was added to the test tube containing 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution, pH 3.5, and 1.5 ml of 0.8% TBA solution. The mixture was diluted to 4 ml with distilled water and heated at 95 °C for 60 min. After cooling on ice, the samples were extracted with 4.0 ml of mixture of n-butanol and pyridine (15:1, v/v). After centrifugation at 3000 rpm for 10 min, the organic phase

was collected and the absorbance was measured at a wavelength of 532 nm. The concentration of 2-thiobarbituric acid was determined using the extinction coefficient of 1.56 × 10⁵ M⁻¹ cm⁻¹. The results were expressed as nanomoles of MDA per mg protein.

2.5.3. Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined for its ability to inhibit the auto-oxidation of pyrogallol according to the method of Del Maestro and McDonald (1986). The reaction mixture (1 ml) consisted of 50 mM Tris (hydroxymethyl) aminomethane (pH 8.2), 1 mM diethylenetriamine penta acetic acid, and 20–50 µl of samples. The reaction was initiated by the addition of pyrogallol (final concentration of 0.2 mM), and the absorbance was measured kinetically at 420 nm (25 °C) for 3 min. The final results were expressed as units/min/mg protein.

2.5.4. Catalase (CAT) activity

Catalase activity was assayed using the method described by Claiborne (1986). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H₂O₂ and a 100 µl sample. The reaction was initiated by the addition of H₂O₂, and the absorbance changes were measured at 240 nm (25 °C) for 30 s. The molar extinction coefficient for H₂O₂ is 43.6 M⁻¹cm⁻¹. The CAT activity was expressed as the unit that is defined as micromole of H₂O₂ consumed per min per mg protein.

2.5.5. Glutathione peroxidase (GSH-Px) activity

GSH-Px was assayed as described by Rotruck et al. (1973). The reaction mixture containing 0.2 ml each of 0.8 mM EDTA, 10 mM sodium azide, 2.5 mM H₂O₂, 0.4 ml of 0.32 M phosphate buffer, and 0.1 ml of homogenate was incubated at 37 °C at different time intervals. The reaction was arrested by adding 0.5 ml of 10% TCA and the tubes were centrifuged at 2000 rpm. To 0.5 ml of supernatant, 4 ml of 0.3 M disodium hydrogen phosphate and 0.5 ml of DTNB (40 mg of DTNB in 100 ml of 1% sodium citrate) were added. The kinetic change was recorded at 420 nm immediately. The GSH-Px activity was expressed as the unit, which is defined as micromole of glutathione oxidized per min per mg protein.

2.5.6. Reduced glutathione (GSH) level

The level of reduced glutathione was measured by the method of Moron et al. (1979). One-milliliter of homogenate was precipitated with 1 ml of 10% TCA and the precipitate was removed by centrifugation. To 0.5 ml of the supernatant, 2 ml of 0.6 mM 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate was added and the total volume was made up to 3 ml with phosphate buffer. The absorbance was read at 412 nm. The level of glutathione was expressed as microgram per g tissue.

2.5.7. Vitamin C (vit C) level

The level of ascorbic acid was estimated by the method of [Omaye et al. \(1979\)](#). To 0.5 ml of homogenate, 0.5 ml of water and 1 ml of 10% TCA were added, mixed thoroughly and centrifuged. To 1 ml of the supernatant, 0.2 ml of DTC (3g of 2,4-dinitrophenyl hydrazine, 0.4 g of thiourea and 0.05 g of copper sulphate were dissolved in 100 ml of 9 N sulfuric acid) reagent was added and incubated at 37 °C for 3 h. Then 1.5 ml of 65% sulfuric acid was added, mixed well and the solutions were allowed to stand at room temperature for another 30 min. Graded amounts of standards were also treated similarly. The color developed was read at 520 nm. The level of ascorbic acid was expressed as microgram per milligram protein.

2.5.8. Vitamin E (vit E) level

The level of vitamin E was estimated by the method of [Desai \(1984\)](#). To 1 ml of homogenate, 1 ml of ethanol was added and thoroughly mixed. Then, 3 ml of petroleum ether was added, shaken rapidly and centrifuged. 2 ml of supernatant was taken and evaporated to dryness. To this, 0.2 ml of 0.2% 4,6-diphenyl-1,10-phenanthroline (in ethanol) was added. The assay mixture was protected from light and 0.2 ml of 0.001 M ferric chloride (in ethanol) was added followed by 0.2 ml of 0.001 M *o*-phosphoric acid (in ethanol). The total volume was made up to 3 ml with ethanol. The color developed was read at 530 nm. The level of vitamin E was expressed as microgram per g tissue.

2.5.9. Protein estimation

The protein content was determined according to the method of [Lowry et al. \(1951\)](#) using bovine serum albumin as a standard.

2.6. Statistical analysis

Values are expressed as mean \pm SD for six rats in each group and significance of the differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparison. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Protein carbonyl content

Oxidative protein damage was measured in different regions of the brains of young and aged rats, as indicated by an increase in protein carbonyl content ([Table 1](#)). In terms of spontaneous carbonyl content, the greatest increase with age was evident for striatum 47%, hippocampus 45%, and cortex 34%, whereas cerebellum 30% and hypothalamus 27% showed a smaller increase. Carbonyl content has been observed to increase in aged rats upon induction with FeCl₂. PCO content was normalized in the *C. asiatica* treated aged rats than in the age-matched controls in both spontaneous as well as stimulated conditions. *C. asiatica* administration did not bring any marked change in the levels of PCO in young rats.

3.2. Lipid peroxidation

The production of thiobarbituric acid reactive substances (TBARS), MDA as an index of lipid peroxidation is presented in [Table 2](#). The results demonstrate that the spontaneous lipid peroxidation in aged brain increased at a rate of 1.6 fold in cortex, 1.5 fold in striatum, 1.7 fold in hippocampus, 1.5 fold in cerebellum and 1.5 fold in hypothalamus as compared

Table 1
Effect of *Centella asiatica* on level of protein carbonyls (nmol/mg protein) in various brain regions of control and experimental rats

Conditions	FeCl ₂ (μM)	Group I	Group II	Group III	Group IV
<i>Cortex</i>					
Spontaneous	–	2.1 \pm 0.19	2.04 \pm 0.2	2.81 \pm 0.27 ^{a,b}	2.21 \pm 0.21 ^c
Stimulated	1.0	6.77 \pm 0.68	6.59 \pm 0.67	9.42 \pm 0.86 ^{a,b}	7.24 \pm 0.71 ^c
<i>Cerebellum</i>					
Spontaneous	–	1.38 \pm 0.14	1.31 \pm 0.13	1.79 \pm 0.16 ^{a,b}	1.41 \pm 0.15 ^c
Stimulated	1.0	5.64 \pm 0.52	5.21 \pm 0.51	7.68 \pm 0.74 ^{a,b}	5.84 \pm 0.59 ^c
<i>Striatum</i>					
Spontaneous	–	2.54 \pm 0.24	2.51 \pm 0.23	3.73 \pm 0.38 ^{a,b}	2.61 \pm 0.24 ^c
Stimulated	1.0	7.18 \pm 0.72	6.96 \pm 0.68	10.84 \pm 1.1 ^{a,b}	7.68 \pm 0.79 ^c
<i>Hypothalamus</i>					
Spontaneous	–	1.42 \pm 0.13	1.38 \pm 0.14	1.81 \pm 0.17 ^{a,b}	1.48 \pm 0.15 ^c
Stimulated	1.0	5.84 \pm 0.59	5.68 \pm 0.53	7.43 \pm 0.73 ^{a,b}	5.63 \pm 0.54 ^c
<i>Hippocampus</i>					
Spontaneous	–	1.29 \pm 0.13	1.27 \pm 0.12	1.87 \pm 0.17 ^{a,b}	1.34 \pm 0.13 ^c
Stimulated	1.0	4.89 \pm 0.51	4.62 \pm 0.43	6.21 \pm 0.61 ^{a,b}	5.24 \pm 0.51 ^{b,c}

Values are expressed as mean \pm SD for six rats in each group.

^a Compared with Group I.

^b Compared with Group II.

^c Compared with Group III significant at $P < 0.05$.

Table 2
Effect of *Centella asiatica* on level of lipid peroxidation (nmols of MDA formed/mg protein) in various brain regions of control and experimental rats

Conditions	FeCl ₂ (μM)	Group I	Group II	Group III	Group IV
<i>Cortex</i>					
Spontaneous	–	0.89 ± 0.07	0.86 ± 0.008	1.46 ± 0.15 ^{a,b}	0.98 ± 0.14 ^c
Stimulated	1.0	2.84 ± 0.29	2.52 ± 0.21	5.23 ± 0.56 ^{a,b}	3.28 ± 0.34 ^{a,b,c}
<i>Cerebellum</i>					
Spontaneous	–	0.46 ± 0.05	0.41 ± 0.03	0.72 ± 0.08 ^{a,b}	0.53 ± 0.05 ^{a,b,c}
Stimulated	1.0	1.87 ± 0.19	1.68 ± 0.16	3.28 ± 0.31 ^{a,b}	2.17 ± 0.22 ^{a,b,c}
<i>Striatum</i>					
Spontaneous	–	0.76 ± 0.08	0.74 ± .07	1.19 ± 0.12 ^{a,b}	0.82 ± 0.08 ^c
Stimulated	1.0	2.14 ± 0.23	2.08 ± 0.21	3.74 ± 0.33 ^{a,b}	2.97 ± 0.23 ^{a,b,c}
<i>Hypothalamus</i>					
Spontaneous	–	0.43 ± 0.04	0.41 ± 0.04	0.66 ± 0.07 ^{a,b}	0.49 ± 0.05 ^{a,b,c}
Stimulated	1.0	1.54 ± 0.16	1.37 ± 0.12	2.63 ± 0.27 ^{a,b}	2.14 ± 0.23 ^{a,b,c}
<i>Hippocampus</i>					
Spontaneous	–	0.48 ± 0.05	0.46 ± 0.04	0.79 ± 0.07 ^{a,b}	0.58 ± 0.06 ^{a,b,c}
Stimulated	1.0	2.18 ± 0.21	1.94 ± 0.18	4.02 ± 0.41 ^{a,b}	2.68 ± 0.27 ^{a,b,c}

Values are expressed as mean ± SD for six rats in each group. Significant at $P < 0.05$.

^a Compared with Group I.

^b Compared with Group II.

^c Compared with Group III.

to young control animals. *C. asiatica* treatment decreased brain regional lipid peroxidation approximately 33% in cortex, 31% in striatum, 27% in hippocampus, 26% in cerebellum and 26% in hypothalamus. In the presence of inducer FeCl₂, a significant ($P < 0.05$) increase in MDA levels was also observed in aged rats when compared to young control animals. Treatment with *C. asiatica* significantly decreased the induced MDA levels in aged rat brain.

3.3. Enzymatic antioxidant

SOD activity in the aged rats was substantially lowered in the brain regions of cortex (44%), hippocampus (39%), striatum (38%), hypothalamus (29%) and cerebellum (28%) when compared to young control rats. CAT activities of the brain regions of old rats were significantly lowered than the young rats. The greater decrease with age was evident for striatum (45%), cortex (38%) and hypothalamus (36%), whereas cerebellum (26%) and hippocampus (25%) showed a smaller decrease. GSH-Px, one of the GSH-depleting enzymes, is a more efficient metabolizer of hydrogen peroxide than catalase. We have found that the activity of GSH-Px was significantly reduced in aged rat brain regions when compared to young control ($P < 0.05$). Administration of *C. asiatica* enhanced the activities of SOD, CAT, GSH-Px in aged rat brain regions when compared with their age matched control rats. *C. asiatica* administration did not produce significant changes in the activities of these enzymes in young rats (Table 3).

3.4. Nonenzymatic antioxidant

Table 4 shows the status of nonenzymatic antioxidants in various brain regions before and after *C. asiatica*

supplementation. There was a significant decline of GSH levels in striatum (33%), hippocampus (31%), cortex (28%), hypothalamus (22%) and cerebellum (19%) in aged rats as compared to young rats. A considerable reduction in the level of ascorbic acid and α -tocopherol by 23%, 21% in striatum, 25%, 28% in hippocampus, 30%, 30% in cortex, 15%, 15% in cerebellum and 16%, 20% in hypothalamus was observed in aged rats compared with young rats. Treatment of aged rats with *C. asiatica* restored the level of glutathione, vitamin C and E to the levels found in young rats. *C. asiatica* supplementation did not bring noticeable alteration in young rats.

4. Discussion

Among various organs the brain and skeletal muscle, constituted mainly of postmitotic cells that reflect ageing changes more markedly than others (Pansarasa et al., 2000). The present findings display an increase in the steady-state level of protein oxidative damage, as indicated by the increase in the concentration of protein carbonyls in aged rats (Table 1). Age-dependent increase in the rate of ROS generation or decline in cellular repair or degradation mechanisms will increase the oxidative load on the cell, resulting in corresponding increase in the concentration of oxidized proteins (Cakatay et al., 2001; Aksenova et al., 1998; Youngman et al., 1992). Aldehydes, such as 4-hydroxy-2-nonenal or malondialdehyde produced during lipid peroxidation can be incorporated into proteins by reaction with either the ϵ -amino moiety of lysine or the sulfhydryl group of cysteine residues to form carbonyl derivatives (Uchida and Stadtman, 1993). Previous studies (Evans et al., 1999; Leeuwenburgh et al., 1999)

Table 3
Effect of *Centella asiatica* on activities of Enzymatic antioxidant in various brain regions of young and aged rats

Parameters	Group I	Group II	Group III	Group IV
<i>Superoxide dismutase (Units/min/mg protein)</i>				
Cortex	0.91 ± 0.08	0.94 ± 0.09	0.51 ± 0.06 ^{a,b}	0.84 ± 0.08 ^c
Cerebellum	1.66 ± 0.16	1.69 ± 0.17	1.19 ± 0.12 ^{a,b}	1.57 ± 0.14 ^c
Striatum	1.54 ± 0.14	1.59 ± 0.16	0.95 ± 0.09 ^{a,b}	1.47 ± 0.15 ^c
Hypothalamus	1.22 ± 0.12	1.26 ± 0.13	0.86 ± 0.08 ^{a,b}	1.15 ± 0.12 ^c
Hippocampus	1.29 ± 0.13	1.34 ± 0.14	0.79 ± 0.08 ^{a,b}	1.15 ± 0.12 ^{b,c}
<i>Catalase (μmol of H₂O₂ consumed/min/mg protein)</i>				
Cortex	2.84 ± 0.27	2.89 ± 0.129	1.75 ± 0.19 ^{a,b}	2.67 ± 0.24 ^{a,c}
Cerebellum	2.36 ± 0.21	2.41 ± 0.25	1.75 ± 0.17 ^{a,b}	2.29 ± 0.23 ^c
Striatum	3.28 ± 0.33	3.32 ± 0.34	2.77 ± 0.24 ^{a,b}	3.19 ± 0.29 ^{a,c}
Hypothalamus	3.91 ± 0.37	3.98 ± 0.41	2.52 ± 0.28 ^{a,b}	3.79 ± 0.36 ^c
Hippocampus	2.16 ± 0.23	2.23 ± 0.21	1.61 ± 0.16 ^{a,b}	2.07 ± 0.21 ^c
<i>Glutathione peroxidase (μmol of GSH oxidized/min/mg protein)</i>				
Cortex	2.01 ± 0.19	2.11 ± 0.22	1.22 ± 0.13 ^{a,b}	1.87 ± 0.19 ^c
Cerebellum	1.84 ± 0.19	1.91 ± 0.18	1.12 ± 0.13 ^{a,b}	1.72 ± 0.18 ^c
Striatum	2.59 ± 0.26	2.64 ± 0.25	1.57 ± 0.16 ^{a,b}	2.34 ± 0.24 ^c
Hypothalamus	1.74 ± 0.16	1.79 ± 0.18	1.18 ± 0.11 ^{a,b}	1.67 ± 0.15 ^c
Hippocampus	1.65 ± 0.15	1.68 ± 0.17	1.09 ± 0.09 ^{a,b}	1.59 ± 0.16 ^c

Values are expressed as mean ± SD for six rats in each group. Significant at $P < 0.05$.

- ^a Compared with Group I.
^b Compared with Group II.
^c Compared with Group III.

demonstrated that both normal rodent brain ageing and normal human brain ageing are associated with an increase in oxidatively modified amino acids, these amino acids and their derivatives are being used as markers to assess oxidative protein damage. Treatment of *C. asiatica* led to a decrease in the level of protein carbonyls in discrete brain regions suggesting the bioactivity of *C. asiatica* to directly react with reactive oxygen species especially effective in scavenging free radicals. As studies have also reported

administration of free radical scavengers to senescent animals reverses protein oxidation in rat brain (Floyd and Hensley, 2000).

Lipid peroxidation is one of the major outcomes of free radical-mediated tissue injury and is an indicator for oxidative damage by a series of chain reactions. In the present study, aged rats showed significant increase in lipid peroxidation as evident by the increase in MDA levels (Table 2). The differences in the levels of lipid peroxidation

Table 4
Effect of *Centella asiatica* on level of non-enzymatic antioxidant in various brain regions of young and aged rats

Parameters	Group I	Group II	Group III	Group IV
<i>Glutathione (μg/g tissue)</i>				
Cortex	0.60 ± 0.06	0.62 ± 0.06	0.44 ± 0.04 ^{a,b}	0.58 ± 0.05 ^c
Cerebellum	0.42 ± 0.04	0.44 ± 0.04	0.34 ± 0.03 ^{a,b}	0.39 ± 0.04 ^c
Striatum	0.44 ± 0.04	0.46 ± 0.04	0.30 ± 0.03 ^{a,b}	0.39 ± 0.04 ^{b,c}
Hypothalamus	0.38 ± 0.04	0.41 ± 0.04	0.30 ± 0.03 ^{a,b}	0.35 ± 0.03 ^{b,c}
Hippocampus	0.48 ± 0.04	0.49 ± 0.05	0.33 ± 0.03 ^{a,b}	0.44 ± 0.04 ^{b,c}
<i>Vitamin C (μg/g tissue)</i>				
Cortex	464 ± 42	471 ± 45	325 ± 38 ^{a,b}	436 ± 41 ^c
Cerebellum	345 ± 32	352 ± 33	293 ± 27 ^{a,b}	336 ± 29 ^c
Striatum	372 ± 26	378 ± 34	287 ± 31 ^{a,b}	360 ± 28 ^c
Hypothalamus	334 ± 31	341 ± 32	279 ± 27 ^{a,b}	330 ± 32 ^c
Hippocampus	391 ± 28	406 ± 38	295 ± 24 ^{a,b}	373 ± 27 ^c
<i>Vitamin E (μg/g tissue)</i>				
Cortex	20.06 ± 2.01	21.4 ± 2.08	14.07 ± 1.42 ^{a,b}	17.29 ± 1.36 ^{b,c}
Cerebellum	13.1 ± 1.28	13.9 ± 1.38	11.14 ± 0.97 ^{a,b}	12.98 ± 1.12 ^c
Striatum	13.8 ± 1.26	14.2 ± 1.38	10.89 ± 0.96 ^{a,b}	12.63 ± 1.06 ^c
Hypothalamus	12.01 ± 1.19	12.96 ± 1.31	9.59 ± 0.87 ^{a,b}	11.42 ± 1.08 ^c
Hippocampus	16.8 ± 1.58	17.6 ± 1.64	12.08 ± 1.27 ^{a,b}	15.8 ± 1.46 ^c

Values are expressed as mean ± SD for six rats in each group. Significant at $P < 0.05$.

- ^a Compared with Group I.
^b Compared with Group II.
^c Compared with Group III.

products observed in various brain regions may be attributed to the differences in their iron content, which influence the generation of reactive oxygen species. Certain brain regions like cortex, striatum and hippocampus are highly enriched with non-heme iron, which is catalytically involved in the production of free radicals (Hill and Switzer, 1984). The peroxidation of membrane lipids eventually leads to loss of membrane integrity and finally to cell death.

The present study showed that *C. asiatica* administration reduces lipid peroxidation in aged rats. The reduction in lipid peroxide levels may be due to the electron and H⁺ donating capacity of flavonoids present in *C. asiatica*, which seem to contribute to the termination of lipid peroxidation chain reaction based on their reducing power. Several studies have shown that flavonoids interact with cell membranes, improving their fluidity, thereby protecting them from lipid peroxidation (Sajja et al., 1995). Our results concord with the earlier work done by Veerendra kumar and Gupta (2002), where it has been reported that *C. asiatica* reduced the lipid peroxidation at a dosage of 300 mg/kg/day in a cognitive deficit rats.

A decline in the activity of SOD is observed in aged rat brain regions (group III) when compared with young rat brain regions (Table 3). In contradiction, reports by Sohal et al. (1994) who inferred that there is no change in antioxidant status (SOD, GPx and CAT) with non-significant alterations. During oxidative stress in the neuronal cells there occurs an increased intracellular calcium ion concentration (Ca²⁺) in the rat brain (Annunziato et al., 2003). This increased intracellular Ca²⁺ can induce the irreversible conversion of xanthine dehydrogenase to xanthine oxidase, which in turn catalyzes the oxidation of xanthine to provide a source of O₂⁻. In addition, auto-oxidation of dopamine in brain could also serve as a source of superoxide anion (Olanow, 1993). These above said mechanism could be the chief possibilities for the reduction in the activity of SOD in aged rats leading to an overload of oxygen radicals. An increase in the activity of SOD was observed in the *C. asiatica* treated (group IV) aged rat brain regions. SOD by catalyzing the removal of superoxide radical (O₂⁻) protects biological membranes and associated structures from free radicals (Carrillo et al., 1992). The phenolic compounds (quercetin and catechins) present in the *C. asiatica* (Zainol et al., 2003) may have different functional property such as scavenging of reactive oxygen species (Hatano et al., 1989), inhibition of the generation of free radicals and chain-breaking activity (Laranjinha et al., 1995). They may act as hydrogen-donating radical scavengers by scavenging lipid alkoxyl and peroxy radical (Bors et al., 1990).

The decreased catalase activity in aged rats (Group III) (Table 3) might be explained by heme oxygenase induction (Schipper, 2000) during ageing, because catalase is a hemeprotein with a prosthetic heme group. The superoxide radical could also inhibit the activity of catalase (Kono and Fridovich, 1982). *C. asiatica* treated aged (group IV) rats

exhibited a significant increase in the activity of catalase when compared to aged control (group III) rats. This indicates that *C. asiatica* scavenges the hydrogen peroxide radicals thereby possibly explaining the results obtained. A report prevails on the effective role of *C. asiatica* as a scavenger of hydrogen peroxide in rat brain (Veerendra kumar and Gupta, 2002).

The decrease in the activity of GSH-Px (Table 3) in aged rat brain regions may be attributed to the decline in glutathione level observed in the present study (Table 4). Increase in substrate (glutathione) availability may be responsible for the increase in the activity of GSH-Px in *C. asiatica* treated aged rats. Veerendra kumar and Gupta (2002) also reported that the *C. asiatica* was found to increase the level of glutathione tripeptide in cognitive deficit rat brain.

The level of glutathione was decreased significantly in aged rat brain regions (Table 4). Our results are in accord with the study of Arivazhagan et al. (2002) indicating the depletion of glutathione in aged rat brain regions. This may affect the ratio of reduced to oxidised glutathione and thereby affecting the total GSH pool of the cell. Diminished GSH status has been linked with normal ageing as well as with neurodegenerative disease (Benzi and Moretti, 1997). Administration of *C. asiatica* was found to enhance the level of glutathione significantly in aged treated rats (group IV) when compared with aged rats (group III). Antioxidant property of *C. asiatica* may possibly be attributed to the phenolic compounds present. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (Rice-Evans et al., 1995).

Ascorbate plays an important role with the lipophilic antioxidant α -tocopherol in protecting the membrane from oxidative stress. Recycling of ascorbic acid requires GSH, which reduces dehydroascorbate to ascorbate (Winkler, 1992). Ascorbate in turn is essential for the recycling of tocopherol radical to tocopherol (Packer et al., 1997). The observed decline in glutathione level (Table 4) may contribute to the decrease in ascorbate as well tocopherol concentration (Table 4) in aged rat brain regions. Treatment with *C. asiatica* enhanced the status of both ascorbic acid and tocopherol in aged rat brain regions. *C. asiatica* has been found to improve the GSH status in the present study, which in turn may improve the levels of ascorbate and tocopherol. Earlier reports suggest that polyphenols may regenerate α -tocopherol through reduction of the α -tocopheroxyl radical (Bors et al., 1990). In the present study, the increase in the level of these antioxidants in drug treated aged rats may be due to the presence of polyphenolic component present in *C. asiatica*. Earlier reports by Arivazhagan et al. (2002) have shown that supplementation of antioxidants in aged rats has a positive effect.

From our findings, we concluded that an increased LPO and PCO level with significant decrease in antioxidant status was observed in all brain regions of aged rats. *C. asiatica* was found to have a challenging role in quenching free

radical induced lipid peroxidation, protein carbonyls and also useful against age-related decline in antioxidant status in aged rat brain regions.

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