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Effect of *Centella asiatica* (Gotu kola) on the antioxidant enzyme activities and glutathione levels in different regions of rat brain during pentylenetetrazole-induced epilepsy

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Abstract

Epilepsy is a convulsive episode and is the most frequent neurodegenerative disorder affecting about 50 million people world-wide. *Centella asiatica* (CA) has been used as a medicine in the Ayurvedic tradition of India for thousands of years. The purpose of the present study is to determine the antiepileptic activity from a medicinal plant CA. The fractionated extracts are found to be active against the pentylenetetrazole (PTZ) induced epileptic rats. The enzymic and non-enzymic antioxidants i.e. reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) superoxide dismutase (SOD), and catalase (CAT) were evaluated in control and experimental groups. The results showed that the CA extracts significantly suppress the (PTZ)-induced epileptic seizures in rats.

Key-Words: *Centella asiatica*, epilepsy, pentylenetetrazole, antioxidants

Introduction

Epilepsy is a common and heterogenous neurological disorder characterized by recurrent spontaneous seizures. It is well known that the epileptic seizures result from excessive discharge in a population of hyperexcitable neurons[1]. Despite the multiple molecular mechanisms have been proposed in generating and spreading epileptic discharges, it has been well established that impaired GABA ergic activity and/ or exaggerated glutamatergic neurotransmission primarily contribute to the various types of epilepsies[2]. Excitotoxicity, the process of neuronal death evolved by glutamate receptor activation, has been hypothesized in both acute and chronic degenerative disorders including epilepsy. It has also been postulated that massive influx of glutamate secreted during epilepsy, activates various free radical generating systems resulting in marked production of oxygen free radicals[3]. Considering the multifactorial neurochemical and neurophysiological malfunctions consequent to epileptic seizures, a few antiepileptic drugs (AEDs) are designed, to mitigate the debilitating aspects of epilepsy[4].

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AED regimens employed in ameliorating seizures generally met with partial success and suffer from substantial problems such as pharmacoresistance and neurotoxic effects[5]. During the past few years, considerable progress has been made towards identifying active factors from indigenous medicinal plants for different human ailments including neurodegenerative disorders such as Alzheimer's disease, parkinsonism etc. Keeping in view of this the present investigation is aimed at studying the modulations of antioxidant metabolism during PTZ-induced epilepsy and antiepileptic treatment with CA.

Material and Methods

Experimental animals

The rats were procured from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the Animal Ethics Committee approval and welfare bearing the CPCSEA 438/01/a/cpcsea/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Epilepsy induced drug

Pentylenetetrazole (PTZ), a convulsing drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of Plant Extracts

The active principles of the leaves of plant were extracted into different solvents, methanol, water, n-hexane, chloroform, ethyl acetate and n-butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants[6, 7]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for our studies.

Administration of tested substance

Each fraction of CA extract (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ at the dose of 60mg/kg body weight[8]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA[7]. The volume of administration was kept at 1ml to the animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/kg bw i.p.) for one week to the experimental animals (Reference control).

Experimental setup

The rats were divided into 8 groups, each group consisted of 6 rats, Group1-Normal saline treated control rats (SC), Group 2-Rats treated with PTZ (Epileptic group), Group 3-Epileptic rats pretreated with n-Hexane extract (nHE+PTZ), Group 4-Epileptic rats pretreated with Chloroform extract (CE+PTZ), Group 5-Epileptic rats pretreated with Ethyl acetate extract (EAE+PTZ), Group 6-Epileptic rats pretreated with n-Butanol extract (nBE+PTZ), Group 7-Epileptic rats pretreated with Aqueous extract (AE+PTZ) and Group 8-Epileptic rats pretreated with Diazepam (DP+PTZ).

Isolation of Tissues

After stipulated duration, the animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons, Medulla (PM) and Hippocampus (HC) were immediately isolated, frozen in liquid nitrogen and were stored at -80^0C until analysis.

Biochemical analysis

Glutathione content was determined according to the method of Theodorus et al[9] (1981). Se-Dependant Glutathione Peroxidase (Se-GSH-Px) was determined by a modified version of Flohe and Gunzler[10] (1984). Glutathione reductase activity was determined by a slightly modified method of Carlberg and Manervik[11] (1985). Glutathione-S- transferase activity was measured with its conventional substrate, 1-chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method of Habig et al[12] (1974). Superoxide dismutase activity was determined according to the method of Misra and Fridovich[13] (1972). Catalase activity was measured by a slightly modified version of Aebi[14] (1984).

Statistical analysis

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 version) for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

Results and Discussion

GSH content

When compared with saline control, PTZ-induced animals had significantly decreased the GSH content in all the brain regions, with highest decrease noted in the cerebral cortex (CC). Pretreatment with CA extracts i.e. n-HE, CE, EAE, n-BE, AE and diazepam (Reference control) were resulted in significantly increased GSH content in all the brain regions (Table 1).

Effect of CA extracts on Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione s-transferase (GST), Superoxide dismutase (SOD) and Catalase (CAT) in different regions of the brain tissue of control and experimental groups

The activities of enzymatic antioxidants in different regions of the brain tissue of control and experimental rats are shown in Tables 2-6. The GPx, GR, GST, SOD

and CAT were significantly reduced in all the brain regions, with the highest decrease were noted in the hippocampus (HC) during PTZ-induced epilepsy (Group II). In the CA extracts treated animals (Groups III -VII) and the diazepam (DP) treated animals (Group VIII); the activities of GPx, GR, GST, SOD and CAT were significantly increased when compared to PTZ bearing animals (Group II).

Oxidants are formed as a normal product of aerobic metabolism and antioxidant defense involves several strategies both enzymatic and non-enzymatic[15]. Chronic oxidative stress has numerous pathological consequences including cancer, arthritis and neurodenerative diseases[16]. Glutathione and associated metabolism is a major mechanism for cellular protection against agents which generate oxidative stress. Glutathione participates in detoxification at several different levels and may scavenge free radicals, reduce peroxides or be conjugated with electrophilic compounds. Thus glutathione provides the cell with multiple defences not only against Reactive oxygen species (ROS) but also against their toxic products.

It is well established that reduced glutathione (GSH) protects against chemically induced injury[17, 18]. The GSH content and the activity levels of Glutathione peroxidase (GPx), Glutathione reductase (GR) and Glutathione s-transferase (GST) were decreased in different regions of brain during PTZ-induced epilepsy. The reduced glutathione metabolism is in agreement with the earlier reports of different models of epilepsy[19]. De Freitas et al[20] have reported similar decrease in GSH levels in rat hippocampus during pilocarpine-induced epilepsy. A wide spread impairment of glutathione system was reported by Mullar et al[21] who have demonstrated decreased glutathione peroxidase in plasma and brain of PTZ-induced epileptic rats. Similar reduction in Glutathione peroxidase activity was also reported in rats treated with PTZ. The depleted levels of Glutathione and subsequent occurrence of oxidative stress have also been demonstrated in *in vitro* glutamate toxicity in neuronal cell line[22]. Reduced microsomal glutathione levels in PTZ-induced epilepsy[23], reduced antioxidant enzyme activities and glutathione levels[24], a generalized diminished antioxidant activity in PTZ-induced epilepsy[25], significant decrease in GSH, Glutathione disulfide (GSSG) in the cerebral cortex of mouse after PTZ-induced seizure have also been reported which are in agreement with the present findings.

The reduced glutathione levels suggest an impairment of cell defence against toxic insult caused due to PTZ and thus result in increased levels of superoxide and hydroxyl radicals. The reduced SOD activity observed in the present study might have lowered the chance of converting oxidized form of glutathione to reduced form of GSH. On par with the diazepam treatment, pre-treatment with different extracts of CA caused reversal of changes in the glutathione metabolism that was impaired during PTZ-induced seizures and exerted their antiepileptic effect by modulating the antioxidant metabolism in different regions of brain. It has also been reported that supplementation of CA significantly protects against arsenic-induced oxidative stress by restoring the blood GSH levels[26]. Gupta et al[27] have also reported that the administration of CA extracts elicited a marked improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and restoration of oxidative stress parameters. Lee et al[28] have reported that the asiatic acid, the major constituent of CA, exerted significant neuroprotective effects on cultured cortical cells by their potentiation of the cellular oxidative defence mechanism. Asiaticoside, another component of CA enhanced induction of antioxidant levels such as SOD, CAT and GPx at an initial stage of wound healing and thus may be regarded as important contributing factors in the healing properties of this substance. The present findings coupled with the earlier reports suggest that the extracts of CA improve the antioxidant ability and offers neuroprotective effect against PTZ-induced epilepsy.

The decreased SOD activity in different regions of brain during PTZ-induced epilepsy suggests failure of dismutation of superoxide anions generated by xanthine oxidase activity. The significant inhibition of SOD activity coupled with increased lipid peroxidation and purine catabolism as observed in the present study suggest the occurrence of oxidative damage during PTZ-induced epilepsy. Similar inhibition of SOD has also been reported in different models of seizures. Wilhelm et al[29] have reported inhibition of Na+, K+ -ATP ases and SOD activities in brains of rats on pilocarpine model of seizures. Obay et al[24] have demonstrated increased lipid peroxidation and decline in antioxidant enzyme activities during PTZ-induced epilepsy. It is speculated that the copious production of glutamate receptors (NMDA and non-NMDA) might have been responsible for spurt in the ROS production and inhibition of SOD activity[30].

In rats treated with different extracts of CA, the SOD activity was enhanced in all the brain regions which suggest the possible involvement of SOD is quenching superoxide anion radical. Consistent with these observations, Shukla et al[31] have reported that Asiaticoside, the anticonvulsant product derived from

Centella asiatica enhanced enzymatic and non-enzymatic antioxidants such as SOD, Catalase, GPx , Vitamin-E and ascorbic acid in induced wound-healing. Gupta and Flora[26], in another study, have concluded that supplementation of *Centella asiatica* significantly protected arsenic-induced oxidative stress. The findings of the present study coupled with the above reports, it can be speculated that the bioactive factors present in different extracts of CA may modulate the pro oxidant / antioxidant balance and pre-treatment with these extracts has a beneficial role is mitigating the debilitating effects of induced epilepsy.

The catalase activity was induced in different regions of rat brain in PTZ-induced epileptic rats after pre-treatment with different extracts of CA. Similar induction of different antioxidant enzymes including catalase has been reported by Jayashree et al[32]. It has also been reported that Asiaticoside, a major constituent of CA, promoted wound-healing by reducing lipid peroxide levels in wounds while it increased enzymatic (SOD, CAT, GPx) and non-enzymatic (Vitamin-E and Ascorbic) antioxidant levels[33]. Significant increase in catalase activity has also been reported after oral treatment with the crude methanol extracts of CA in lymphoma-bearing mice[32]. Decreased lipid peroxidation and increased catalase activity have been recorded in erythrocytes of CA treated rats during H_2O_2-induced oxidative stress[34]. Improved catalase and SOD activity levels have also been demonstrated in monosodium glutamate treated rats after pre-treatment with chloroform, methanolic extract of CA[35].

From the present findings coupled with the earlier reports, it is obvious that the bioactive factors present in *Centella asiatica* stimulated the antioxidant enzymes such as GPx, GR, GST, SOD and CAT in order to neutralize the oxidant radicals and lipid peroxides generated during induced epilepsy. Further more, extracts of CA significantly attenuated the excitotoxic effects of glutamate a major abundant excitatory neurotransmitter that is produced in excess during epileptogenesis. The present data also suggest that the CA extracts modulate the pro oxidant / antioxidant balance and reduce the seizure manifestations and accompanying biochemical changes and highlights the possible role of antioxidant therapy as adjuncts to antiepileptic drugs for better seizure control.

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Table 1: Alterations in the content of Glutathione (GSH) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	2.023	0.829*	3.660*	3.346*	2.643*	3.306*	2.840*	3.548*
	±0.011	±0.007	±0.869	±1.554	±0.023	±0.017	±0.010	±0.012
		(-59.01)	(80.91)	(65.39)	(30.63)	(63.43)	(40.40)	(75.39)
CB	5.071	3.457*	7.333*	7.639*	6.393	6.036	6.901*	6.486
	±0.037	±0.017	±0.025	±0.024	±0.020	±0.028	±0.036	±0.023
		(-31.83)	(44.60)	(50.63)	(26.07)	(19.02)	(36.09)	(27.90)
HC	3.648	1.595*	4.536	5.710*	5.364*	6.420*	4.352	5.547*
	±0.017	±0.016	±0.012	±0.989	±0.016	±0.022	±0.016	±0.022
		(-56.35)	(24.14)	(58.45)	(46.80)	(75.69)	(19.11)	(51.79)
PM	3.423	2.094*	6.048*	4.332	5.949*	4.902*	4.613*	5.154*
	±0.032	±0.018	±0.018	±0.021	±0.019	±0.023	±0.014	±0.015
		(-38.83)	(76.69)	(26.56)	(73.80)	(43.19)	(34.76)	(50.57)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as nano moles of GSH formed/ gm wet wt. of tissue
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 2: Alterations in the activity of Glutathione peroxidase (GPx) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	1.315	1.237	1.543*	1.510*	1.451*	1.403	1.363	1.553*
	±0.017	±0.024	±0.023	±0.016	±0.024	±0.011	±0.028	±0.017
		(-5.95)	(17.36)	(14.79)	(10.36)	(6.68)	(3.63)	(18.12)
CB	1.504	1.405	1.703*	1.664*	1.651	1.591	1.541	1.724*
	±0.033	±0.007	±0.015	±0.020	±0.009	±0.029	±0.007	±0.021
		(-6.59)	(13.26)	(10.65)	(9.80)	(5.78)	(2.48)	(14.65)
HC	1.252	1.141	1.440*	1.332	1.366	1.351	1.342	1.444*
	±0.021	±0.011	±0.012	±0.007	±0.022	±0.020	±0.029	±0.016
		(-8.84)	(15.03)	(6.40)	(9.07)	(7.89)	(7.18)	(15.36)
PM	1.043	0.942	1.294*	1.222*	1.188*	1.143	1.093	1.239*
	±0.023	±0.016	±0.030	±0.017	±0.019	±0.015	±0.015	±0.019
		(-9.67)	(24.01)	(17.12)	(13.88)	(9.56)	(4.78)	(18.77)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 3: Alterations in the activity of Glutathione reductase (GR) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	1.759	1.102*	2.296*	2.387*	2.349*	2.348*	2.312*	2.412*
	±0.013	±0.247	±0.205	±0.203	±0.107	±0.245	±0.241	±0.121
		(-37.34)	(30.53)	(35.71)	(33.54)	(33.50)	(31.44)	(37.10)
CB	5.357	3.715*	7.520*	6.659*	7.603*	6.852*	6.984*	7.175*
	±0.018	±0.146	±0.179	±0.210	±0.128	±0.230	±0.121	±0.245
		(-30.47)	(40.74)	(24.63)	(42.29)	(28.25)	(30.71)	(34.29)
HC	2.930	1.574*	4.132*	3.878*	4.222*	4.084*	4.076*	4.331*
	±0.020	±0.247	±0.145	±0.128	±0.249	±0.120	±0.246	±0.121
		(-46.29)	(41.02)	(32.36)	(44.10)	(39.39)	(39.13)	(47.82)
PM	3.695	2.366*	4.604*	4.743*	4.842*	4.804*	4.793*	4.885*
	±0.032	±0.124	±0.012	±0.069	±0.078	±0.089	±0.099	±0.240
		(-35.97)	(24.60)	(28.36)	(31.04)	(30.01)	(29.72)	(32.20)

All the values are mean, ±SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 4: Alterations in the activity of Glutathione s-transferase (GST) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	4.320	2.865	5.718	5.592	5.935	5.285	5.642	6.150
	±0.018	±0.027	±0.028	±0.021	±0.077	±0.047	±0.038	±0.016
		(-33.68)	(32.36)	(29.44)	(37.39)	(22.33)	(30.60)	(42.37)
CB	1.907	1.294	2.450	2.415	2.387	2.426	2.515	2.648
	±0.020	±0.102	±0.060	±0.086	±0.083	±0.120	±0.059	±0.060
		(-32.13)	(28.45)	(26.62)	(25.15)	(27.21)	(31.86)	(38.81)
HC	0.731	0.461	0.988	1.009	1.065	1.021	1.088	1.066
	±0.011	±0.060	±0.056	±0.029	±0.058	±0.064	±0.027	±0.075
		(-36.88)	(35.16)	(38.07)	(45.65)	(39.64)	(48.86)	(45.88)
PM	2.672	1.831	3.427	3.581	3.825	3.616	3.862	3.862
	±0.032	±0.037	±0.068	±0.056	±0.031	±0.047	±0.074	±0.080
		(-31.44)	(28.28)	(34.05)	(43.16)	(35.36)	(44.57)	(44.55)

Values in ‘ ( ) ‘ parenthesis are % change over saline control
* Values All the values are mean, ± SE of six individual observations.
are significant at $P<0.05$ in Scheffe test.
Values are expressed as µ moles of thioether formed/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 5: Alterations in the activity of superoxide dismutase (SOD) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	13.242	8.621*	15.963*	15.915*	16.003*	16.161*	16.066*	16.038*
	±0.398	±0.175	±0.539	±0.554	±0.335	±0.385	±0.217	±0.261
		(-34.89)	(20.54)	(20.18)	(20.85)	(22.04)	(21.32)	(21.11)
CB	18.258	10.579*	23.27*	23.072*	23.523*	24.082*	23.744*	24.127*
	±0.418	±0.304	±0.611	±0.303	±0.304	±0.143	±0.435	±0.356
		(-42.05)	(27.47)	(26.36)	(28.83)	(31.89)	(30.04)	(32.14)
HC	30.967	15.946*	42.647*	40.076*	43.109*	43.176*	41.697*	41.548*
	±0.322	±0.323	±0.260	±0.524	±0.237	±0.317	±0.588	±0.250
		(-48.50)	(37.71)	(29.41)	(39.20)	(39.42)	(34.64)	(34.16)
PM	23.838	13.592*	31.668*	29.014*	31.406*	32.223*	29.251*	31.875*
	±0.497	±0.223	±0.212	±0.364	±0.112	±0.231	±0.137	±0.329
		(-42.98)	(32.84)	(21.71)	(31.74)	(35.17)	(22.70)	(33.71)

All the values are mean, ± SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at P<0.05 in Scheffe test.
Values are expressed as µ moles of epinephrine oxidized/ mg of protein/min
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Table 6: Alterations in the activity of Catalase (CAT) in different regions of rat brain during PTZ-induced epilepsy and pre-treatment with different extracts of *Centella asiatica*

BRAIN REGION	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	DP+PTZ
CC	0.425	0.286*	0.527*	0.564*	0.562*	0.622*	0.615*	0.615*
	±0.104	±0.071	±0.125	±0.146	±0.131	±0.103	±0.149	±0.103
		(-39.21)	(35.60)	(30.37)	(29.99)	(32.63)	(39.71)	(34.52)
CB	0.528	0.369*	0.775*	0.797*	0.624*	0.723*	0.678*	0.601*
	±0.139	±0.108	±0.097	±0.111	±0.078	±0.055	±0.080	±0.124
		(-31.73)	(34.80)	(40.84)	(26.24)	(29.18)	(36.28)	(28.08)
HC	0.626	0.292*	0.771*	0.831*	0.857*	0.844*	0.846*	0.838*
	±0.059	±0.070	±0.031	±0.092	±0.085	±0.062	±0.152	±0.099
		(-46.28)	(44.34)	(51.05)	(47.04)	(45.98)	(49.30)	(52.04)
PM	0.716	0.375*	0.815*	0.847*	0.922*	0.831*	0.849*	0.974*
	±0.112	±0.124	±0.085	±0.080	±0.066	±0.046	±0.063	±0.152
		(-38.81)	(36.74)	(26.59)	(43.85)	(43.23)	(34.79)	(50.62)

All the values are mean, ± SE of six individual observations.
Values in ' ( ) ' parenthesis are % change over saline control
* Values are significant at $P<0.05$ in Scheffe test.
Values are expressed as µ moles H_2O_2 hydrolyzed/mg protein/min.
Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)