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ORIGINAL ARTICLE

Dietary Supplementation of *Hemidesmus indicus* and Swimming Exercise Attenuates Oxidative Stress in the Rat Brain

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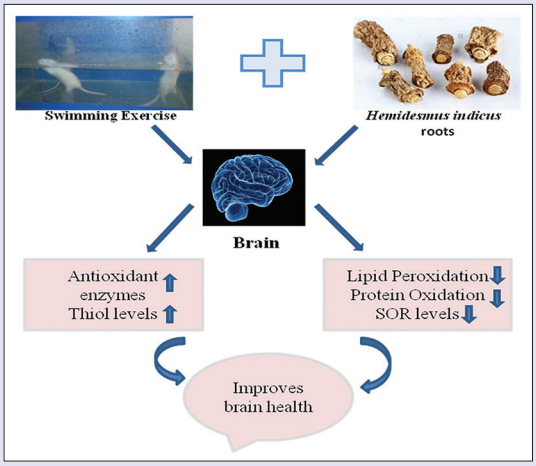
ABSTRACT

Background: In the current investigation, we explored the impact of swimming training and dietary fortification of *Hemidesmus indicus* (HI) extract on markers of oxidative stress (OS) and antioxidant capacity in the middle-aged Wistar rats. **Materials and Methods:** Male rats received oral supplementation of HI at a dosage of 50 and 100 mg/kg B.W and swim trained for 30 min/day with 3% intensity, 6 days/week for a duration of 84 days. **Results:** Training and dietary intake of 100 mg/Kg B.W HI extract enhanced antioxidant enzyme activities in the discrete regions of the brain. The OS indicators were attenuated in response to single or combined interventions. The levels of thiols were significantly upregulated in SW-T (+HI2) group. **Conclusion:** Our findings suggest that both interventions improve antioxidant status, by attenuating the OS markers, which can be used as a therapeutic strategy in preventing age-associated neurological disorders.

Key words: Antioxidant enzymes, dietary supplementation, *Hemidesmus indicus,* oxidative stress, swimming exercise

SUMMARY

- The combined interventions of exercise and the diet on antioxidant status of the brain were evaluated.
- The exercise and diet were found to be potent in ameliorating the antioxidant status of the brain.
- Our investigation demonstrated that that nutrition and physical activity play a significant role in retaining brain health.



Abbreviations used: HI: *Hemidesmus indicus;* CC: Cerebral cortex; HC: Hippocampus; CB: Cerebellum; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; PC: Protein carbonyls; MDA: Malondialdehyde; SOR: Superoxide radical; T-SH: Total thiols; P-SH: Protein thiols; NP-SH: Non-protein thiols.

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INTRODUCTION

Oxidative stress (OS) has been associated with the pathophysiology of various neurological ailments.[1] OS occurs as an outcome of disparity between oxidants and antioxidant system.[2] Reactive oxygen species (ROS) are the end products of cellular processes which causes damage to biomolecules such as proteins, membrane lipids, and DNA. Oxidative breakdown of proteins is reflected by overexpression of protein carbonyls (PC) levels and reduced levels of protein thiols (P-SH).[3] Reactions of ROS with amino acid side chains (histidine, proline, lysine, threonine, and arginine) leads to the formation of carbonyl products which are employed as OS markers.[4] Protein carbonylation in the brain is associated with the pathophysiology and development of several neuronal disorders.

The central nervous system is highly susceptible to OS when compared to other tissues because of its high oxygen requirement, higher amounts of peroxidizable lipids, and modest levels of antioxidants.[5] Antioxidant enzyme defense constitutes the chief protection to the brain against radicals by the action of superoxide dismutase (SOD), and peroxides through catalase (CAT) and glutathione peroxidase (GPx). The enzymatic defense systems are modulated by several factors such as aging, physical activity, and diet.[6-8] Regular physical exercise is an important element for a healthy life.[9] Studies in humans[10,11] and animals[12,13] have reported that physical activity shields neurons from several brain abuses, stimulates neurons, initiates neurogenesis, and enhances cognition.[9]

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In addition to exercise, diet also plays a remarkable role in brain functioning.[14,15] Phytochemicals have been proven to be effective free radical scavengers because of their potent antioxidant activity.[16] Recent studies have been carried out on indigenous medicines exhibiting neuroprotective activities.[17] *Hemidesmus indicus* (L) R. Br.(HI) is widely called as Indian sarsaparilla. It is a twining shrub which belongs to the family *Apocynaceae*. HI is usually employed in traditional Indian medicine for the management of various ailments. The roots possess a broad spectrum of bioactive molecules such as α-amyrin, β-amyrin, 2-hydroxy-4-methoxy-benzoic acid, lupeol, hemidesmin 1, hemidesmin 2, and some triterpenes which contribute to its therapeutic activity.[18,19] Earlier studies from our laboratory reported that phenolic and flavonoid content in the aqueous root extract corresponds to its potent antioxidant activity.[20] Studies have also demonstrated that HI root extract exhibits antidiabetic,[21] hepatoprotective,[22] and neuroprotective[23] properties.

However, limited reports are available with respect to the synergistic effect of exercise and dietary supplementation of HI extract in the brain. Therefore, we hypothesized to investigate the effect of two interventions in ameliorating OS in the cerebral cortex (CC), hippocampus (HC), and cerebellum (CB) regions of the rat brain. The hypothesis was examined by evaluating the antioxidant status and OS parameters.

MATERIALS AND METHODS

Chemicals

Epinephrine, glutathione reductase, reduced glutathione (GSH), guanidine hydrochloride, t-butyl hydroperoxide, nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide, 2,4-dinitrophenylhydrazine (DNPH), reduced nicotinamide dinucleotide phosphate (NADPH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and phenylmethylsulfonyl fluoride were procured from Sigma-Aldrich (St. Louis, MO, USA). Other analytical grade reagents and solvents were obtained from local companies.

Aqueous root extract preparation

The HI roots were obtained from B.R. Hills, Karnataka, India. The plant material was authenticated in the Department of Botany, Bangalore University, Bengaluru, and a specimen was deposited in the herbarium (BUB, NO.2224). The roots were washed with water, and the outer layer was separated, dehydrated in the oven at 40°C, and powdered finely. The extract was made by solubilizing 100 g of the powder into 1 liter of water maintained at 50°C. After 24 h, it was filtered using Whatman paper, and the obtained filtrate was freeze-dried and weighed (16% yield) and preserved at 4°C for further use.[15]

Animal maintenance

Male adult *Wistar* strain rats of 2 months old were purchased from Venkateshwara Enterprises, Bengaluru and maintained till they attained 12 months of age. Three rats were housed per cage, maintained at 28°C ± 1°C temperature, relative humidity of 77 ± 1% and 12 h-dark/light cycle. They had free access to standard feed (Amruth Feeds, India) and tap water *ad lib*. The designed protocol was accepted by Institutional Animal Ethical Committee (IAEC) (BUB/IAEC/TRK/05/2015) and pertaining to the instructions prescribed by the committee for the purpose of control and supervision of experiments on animals.

Dosage fixation

Different doses of HI extract (50, 100, 150, and 200 mg/kg BW) were screened to determine the dose-dependent effect in discrete brain regions of the rat. The effective doses were assessed based on the antioxidant enzyme levels and OS markers. HI extract of 50 and 100 mg/kg BW was found to be more prominent in attenuating the OS markers and enhancing the antioxidant status. Hence, these doses were fixed for further studies.

Experimental design

The rats were distributed into three swimming training groups ($n = 8$) – (i) trainees with normal diet (SW-T[N]), (ii) trainees given dietary supplementation of HI extract (50 mg/kg BW [SW-T(+HI_1)]), (iii) trainees on a dietary supplementation of HI extract (100 mg/kg BW [SW-T(+HI_2)])-and three sedentary groups ($n = 8$), (iv) sedentaries with normal diet (SE-C[N]), (v) sedentaries supplemented with HI extract (50 mg/kg BW) (SE-C[+HI_1]), and (vi) sedentaries fed with HI extract (100 mg/kg BW) (SE-C[+HI_2]).

Rats were administered with HI extract 50 and 100 mg/kg BW, respectively, using the intragastric tube daily for a duration of 84 days. Along with the fortification of HI extract, group (i), (ii), and (iii) rats were exposed to swimming exercise.

Exercise training

Swimming exercise training was performed according to the procedure of Anand *et al*.[24] Rats were allowed to swim in a rectangular glass tank (77 cm × 38 cm × 39 cm) with water filled to a height of 22 cm, maintained at 32°C ± 1°C with 3% body weight load knotted to their tails. Initially, the rats were trained for 5 min/day with a gradual increment to 30 min/day and subsequently for 30 min/day, 6 days in a week for a total training regimen of 84 days. SE-Cs were confined to cage activities.

Preparation of tissue extract

Following the experimental paradigm, the rats were subjected to CO_2 asphyxiation. The brain was excised, and the three regions (CC, HC, and CB) were separated and washed with chilled saline. The brain regions were weighed and preserved at −180°C for further analyses. The homogenates were prepared using phosphate buffer (50 mM, pH 7.4) and were utilized for the quantification of malondialdehyde (MDA), superoxide radical (SOR), and thiols. The remaining homogenates were centrifuged at 2000 ×*g* for 10 min at 4°C (Superspin-RV/FM, Plastocrafts). The obtained supernatant was employed for the estimation of antioxidant enzymes PC.

Activities of antioxidant enzymes

SOD activity was assessed following the protocol of Misra and Fridovich.[25] To 880 µL of carbonate buffer (0.05 M, pH 10.2) and ethylenediamine tetraacetic acid (0.1 mM), 100 µL of supernatant was added. Following this, 20 µL of epinephrine (30 mM) was added to the above mixture and absorbance was monitored at 480 nm in a spectrophotometer (Model SL 159, ELICO) for 5 min. The enzyme concentration which resulted in 50% epinephrine auto-oxidation inhibition was defined as one unit.

The procedure of Aebi.[26] was followed for the estimation of CAT activity. 100 µL of the supernatant and 100 µL of absolute alcohol were mixed and incubated at 4°C for 30 min followed by the addition of 10 µL of triton X-100. From the above solution, 10 microlitre was taken, and 240 microlitre of phosphate buffer, followed by 250 microlitre of H_2O_2 (0.066 M) was added, mixed and the reduction in absorbance was read at 240 nm for 60 s. An extinction coefficient of 43.6 M/cm was taken for the calculation of enzyme activity. One unit was equivalent to the µmoles of H_2O_2 degraded/min/mg protein.

The activity of GPx was assessed following the protocol of Flohé and Günzler.[27] Briefly, 500 µL of phosphate buffer, 100 µL of GSH (0.01 M), 100 µL of NADPH (1.5 mM) and 100 µL of glutathione reductase were

added to a cuvette. To the above mixture, 100 µL of supernatant was added and kept for 10 min at 37°C. 450 µL from the above reaction mixture was taken, and 50 µL of t-butyl hydroperoxide (12 mM) was mixed, and absorbance was read for 3 min at 340 nm. The molar absorptivity of 6.22 × $10^3M^{-1}cm^{-1}$ was employed for the determination of activity. One unit of enzyme activity is equivalent to mM NADPH oxidized/min/mg protein.

Oxidative stress markers

Estimation of superoxide radical

SOR levels were assessed according to the protocol of Das *et al.*[28] 200 µL homogenate was mixed with 80 µL of NBT (0.1%) and incubated at 37°C for 1 h in an oscillating water bath. The assay was terminated, and removal of the reduced NBT was processed by centrifuging the samples for 10 min at 200 ×*g* and then suspending the pellets using glacial acetic acid. The absorbance was documented at 560 nm and converted to µmoles diformazan using nitroblue diformazan standard curve. The findings were reported as µmoles diformazan/mg protein.

Quantification of protein oxidation

The protocol of Levine and Stadtman[4] was adopted for the determination of PC levels. 100 µL of supernatant and 0.5 mL of DNPH (10 mM in 2 M HCl) were incubated in the dark for 60 min. 0.5 mL of Trichloroacetic acid (TCA) (20%) was added and then centrifuged at 10,000 ×*g* at 4°C for 3 min to precipitate proteins. The obtained pellet was treated with ethyl acetate/ethanol (1:1) twice by centrifugation (3400 ×*g* for 5 min) to eliminate excess DNPH. The pellet was solubilized in guanidine hydrochloride (6 M). Absorbance was recorded at 370 nm. The concentration of carbonyls was estimated from the extinction coefficient of 22,000$M^{-1}CM^{-1}$ and represented as nmol/mg protein.

Quantification of lipid peroxidation

MDA levels were determined following the protocol of Ohkawa *et al.*[29] using standard TMP (1,1,3,3, tetramethoxypropane). 100 µL of homogenate, 1.5 mL of 20% acetic acid, 200 µL of sodium dodecyl sulfate (8.1%), and 1.5 mL of 0.8% TBA solution were mixed, and the volume was made to 4 mL with d.w. This mixture was kept at 100°C for 60 min, cooled, and 1 mL of d.w, 5 mL butanol:pyridine (15:1) were added. Following this, the mixture was mixed and centrifuged for 10 min at 4000 ×*g*. The orange layer obtained was read at 532 nm.

Measurement of total thiols, protein thiols and non-protein thiol levels

The thiols were measured using the procedure of Sedlak and Lindsay.[30] To estimate total thiols (T-SH), 250 µL of the homogenate, 750 µL of 0.2 M Tris buffer (pH 8.2) and 50 µL of 0.01 M DTNB were mixed together, and the total volume was made to 5 mL with methanol. A sample blank and a reagent blank were prepared. The resultant mixture was centrifuged for 15 min at 3000 ×*g*, and the optical density of the supernatants was recorded at 412 nm. 13,100/M/cm molar extinction coefficient was used to quantify T-SH and non-protein thiols (NP-SH) levels.

For estimation of NP-SH, 250 µL of the homogenates, 200 µL d.w. and 50 µL of 50% TCA were mixed. The tubes were shaken well and centrifuged for 15 min at 3000 ×*g*. 200 µL of the supernatant, 10 µL of DTNB, and 400 µL of 0.4 M Tris buffer (pH 8.9) were mixed and the absorbance was recorded at 412 nm. The P-SH were obtained by deducting the NP-SH from T-SH levels.

Protein estimation

Protein concentration was determined by the procedure of Lowry *et al.*[31] using bovine serum albumin.

Statistical analysis

The results were represented as mean ± standard error and analyzed with a two-factorial analysis of variance between regions and groups. "*F*" ratios significant between the group means were further analyzed using Tukey's test. Probability (*P*) <0.05 was considered as statistically significant. All statistical analyses were executed using GraphPad PRISM 6.7 software (San Diego, CA, USA).

RESULTS

Body weight changes

A significant reduction in body weights was noticed in swim trainees and supplemented trainees, as represented in Table 1. However, the maximum decrease in body weight was evident in SW-T(+HI_2) group by 3% during the 12th week of training with respect to the sedentary controls.

Antioxidant enzymes

The levels of SOD, CAT, and GPx were remarkably upregulated in supplemented trainees and supplemented groups. However, SW-T(+HI_2) group showed higher enzyme activity in all the three regions compared to other experimental groups [Table 2]. A notable feature in the CAT activity was that significant variations were observed between the regions.

Markers of oxidative stress

SOR, a major free radical generated during physiological reactions, were found to be increased in the HC relative to other regions. SOR levels were lowered significantly in all the experimental groups except the SW-T(N) group. The extent of decrease being greater in SW-T(+HI_2) by 22%, 27%, and 28% in CC, HC, and CB, respectively, over their sedentary controls [Figure 1a].

The changes in PC content are depicted in Figure 1b. There was a noticeable reduction in PC content in the supplemented trainees and SE-C(+HI_2) compared to SE-C (N). A remarkable feature is that insignificant variations were observed among the regions as well as within the supplemented trainees and sedentaries.

The MDA content, a marker of lipid peroxidation, exhibited higher levels in the CC as represented in Figure 1c. The MDA content was decreased maximally in SW-T(+HI_2) (CC- 41%, HC- 40% and CB- 49%) and SE-C(+HI_2) groups (CC- 25%, HC- 34% and CB- 42%) respectively over their sedentary controls.

Table 1: Changes in body weight (g) as a function of exercise and diet

Groups	3rd week	6th week	9th week	12th week
SE-C (N)	351.00±2.02	356.00±2.60*	359.66±2.60*	365.66±3.75*
SW-T (N)	347.33±1.73	348.66±2.30	351.66±3.20	355.00±3.46#
SE-C(+HI_1)	347.66±2.33	351.66±2.90	353.66±2.90	357.66±4.05#
SW-T(+HI_1)	347.66±2.60	350.66±3.21	351.66±2.60	354.66±4.33#
SE-C(+HI_2)	350.00±2.30	352.66±2.88	355.66±2.88	358.66±3.46#
SW-T(+HI_2)	348.33±3.48	350.66±2.90	353.00±3.48	355.66±3.48#

*Significance in comparison with the 3rd week; #Significance between the sedentary control and experimental groups. Values are expressed as mean±SE of eight animals/group and were analyzed by one-way ANOVA followed by Tukey's test. *,#*P*<0.05 was considered significant. ANOVA: Analysis of variance; SE: Standard error

Table 2: Antioxidant enzyme activities in discrete brain regions of the rats

Parameters	Groups					
	SE-C (N)	SW-T (N)	SE-C(+HI_1)	SW-T(+HI_1)	SE-C(+HI_2)	SW-T(+HI_2)
SOD						
CC	13.01±0.61	16.15±0.98	17.74±1.42*	18.07±1.60*	21.92±1.36*	20.92±0.60*
HC	14.49±0.33	16.96±1.52	20.04±1.22	20.69±1.16	22.26±1.99	23.16±0.83
CB	13.89±1.21	16.12±1.12	18.07±1.92	17.31±0.85	20.37±2.50	21.30±0.77
CAT						
CC	17.31±0.80	24.22±1.29*	31.78±1.33*	39.32±1.18*	39.81±1.28*	39.96±1.13*
HC	18.12.±0.89	23.57±1.22	38.38±0.61	41.23±0.70	42.96±1.56	43.18±1.80#
CB	17.40±0.58	22.15±0.71	37.72±0.56	38.91±0.57	37.87±0.57	38.63±0.94
GPx						
CC	12.11±0.74	17.23±0.84*	23.25±1.62*	24.22±0.77*	24.48±0.93*	27.45±1.38*
HC	14.17±0.68	18.17±0.50	22.54±0.87	21.37±1.76	23.05±1.53	23.22±0.96
CB	17.19±0.87	17.61±0.64	22.09±0.89	20.83±1.95	22.24±1.21	23.44±0.84

*Significance between the sedentary control and experimental groups; #Comparison between the regions. Values are expressed as mean±SE and were analyzed by two-way ANOVA followed by Tukey's test. *,#P<0.05 was considered as statistically significant. Units - SOD: Units/mg protein; CAT: Micromoles of H_2O_2 consumed/min/mg protein; GPx: Nmoles of NADPH oxidized/min/mg protien. ANOVA: Analysis of variance; SE: Standard error; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; CC: Cerebral cortex; HC: Hippocampus; CB: Cerebellum; NAPDH: Nicotinamide dinucleotide phosphate

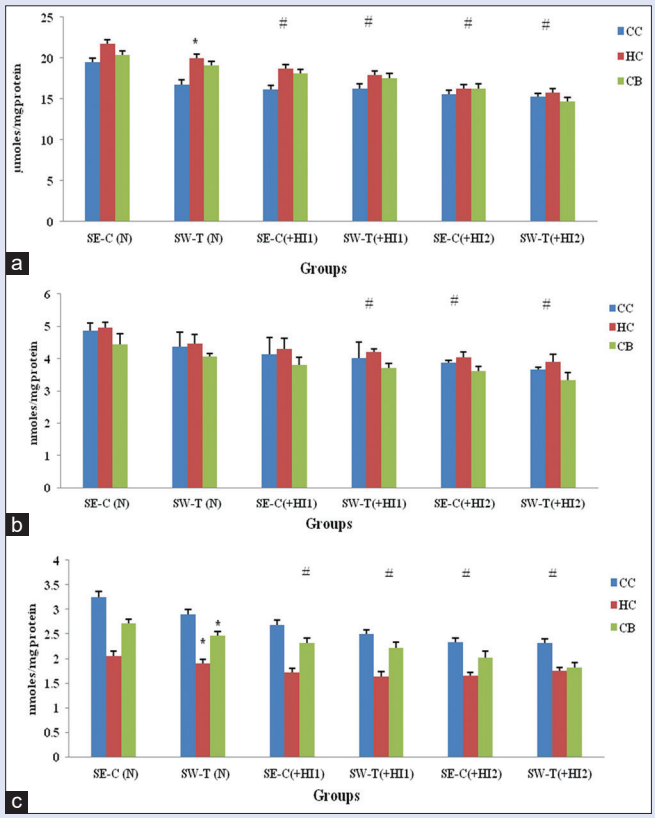

Figure 1: The levels of (a) superoxide radical, (b) protein carbonyls, and (c) malondialdehyde in discrete brain regions of the rat. Values are expressed as mean ± standard error of eight animals/group and were analyzed by two-way analysis of variance followed by Tukey's test. *,#P < 0.05 was considered as statistically significant. *The comparison of cerebral cortex with hippocampus and cerebellum. #The comparison of sedentary control with experimental groups

Thiols

The T-SH, NP-SH, and P-SH levels in the different brain regions are depicted in Table 3. T-SH content was considerably enhanced in all the groups with HC exhibiting higher levels compared to other regions. SE-C(+HI_2) and SW-T(+HI_2) groups exhibited a maximum elevation in T-SH levels in all the regions over their sedentary counterparts. Region-specific alterations were evident in NP-SH levels and revealed a similar trend as that of T-SH levels. Maximum increase was observed in SW-T(+HI_2) by 12% - CC, 20% - HC and 8% - CB relative to their SE-C (N). The P-SH levels were also significantly enhanced across all the experimental groups. HC and CB regions showed higher content compared to CC. The SE-C(+HI_2) and SW-T(+HI_2) groups revealed higher levels with respect to unsupplemented sedentaries.

DISCUSSION

Diet and exercise are important lifestyle factors in maintaining cognitive health and psychological wellbeing. Studies have shown that intake of polyphenols rich diet can help healthy aging, thus delaying the cognition, and extending the life span.[32,33] The roots of HI is a rich source of nutraceuticals, and there are reports on the hepatoprotective effect of HI extract in rats.[34,35] However, the neuroprotective and cognitive ability of the HI extract has not been carried out. Therefore, the current investigation was aimed to examine the combinational effect of exercise, and supplementation of HI extract in ameliorating OS in the discrete brain regions of the rat.

Swimming was selected as an appropriate model of physical activity as rodents are inborn swimmers, and there is a less mechanical injury with better distribution of blood and insignificant variations in cardiac output.[33] In this study, body weight is significantly decreased in swimming and supplemented trainees, suggesting that diet and exercise intervention were effective in lowering the bodyweight of rats. The results are in concordance with the earlier studies of Ravikiran *et al.*[15] and Abhijit *et al.*[36]

Cells have endogenous antioxidant defense systems (SOD, CAT, and GPx) which protect the cells from ROS. SOD removes SORs, whereas CAT and GPx decompose H_2O_2 to O_2 and H_2O. We observed the increased activity of antioxidant enzymes in all the experimental groups and regions. The upregulated enzyme activities in the supplemented and trained groups revealed the combined interventions of exercise and diet in maintaining the brain antioxidant defense system. Region-specific variations were not evident for CAT and GPx activity. Acikgoz *et al.*[5] and Ravikiran *et al.*[15] also found no significant changes in the activities of SOD and GPx between the different brain regions subjected to exercise training, indicating that the activity of these enzymes may vary due to the type of exercise, its duration, and intensity, as well as dosage of supplementation.

Table 3: The level of total thiols, non-protein thiols, and protein thiols in discrete brain regions of the rats

Parameters	Groups					
	SE-C (N)	SW-T (N)	SE-C(+HI_1)	SW-T(+HI_1)	SE-C(+HI_2)	SW-T(+HI_2)
T-SH						
CC	104.49±4.40	109.38±4.17*	114.54±4.12*	120.39±3.54*	122.08±3.55*	122.26±4.69*
HC	103.63±3.45	118.48±3.12	129.18±3.23	130.41±4.02	133.51±3.04	139.64±3.15#
CB	112.34±3.36	113.72±3.45	115.37±3.11	118.50±3.64	120.34±3.23	120.79±4.03
NP-SH						
CC	64.05±3.96	65.56±3.66	66.56±3.05*	70.40±3.01*	71.49±2.98*	72.18±2.04*
HC	66.49±2.78	67.17±3.01	75.36±2.67	75.53±3.03	78.21±2.44	82.13±2.78#
CB	64.30±2.68	64.88±2.78	65.49±2.35	68.17±2.45	69.31±2.49	69.65±1.97
P-SH						
CC	40.44±2.75	43.82±7.09*	47.98±10.27*	49.99±8.19*	50.59±3.78*	50.08±1.10*
HC	37.14±2.87	51.31±3.41	53.65±06.08	54.88±10.30	55.30±10.20	57.51±6.83#
CB	48.04±6.63	48.84±11.4	49.88±10.70	50.33±0.90	51.03±2.49	51.14±1.76#

*Significance between the sedentary control and experimental groups; #Comparison between the regions. Values are expressed as mean±SE of eight animals/group and were analyzed by two-way ANOVA followed by Tukey's test. *,#$P<0.05$ was considered significant. Units: nmol/mg protein. ANOVA: Analysis of variance; SE: Standard error; T-SH: Total thiols; NP-SH: Non-protein thiols; P-SH: Protein Thiols; CC: Cerebral cortex; HC: Hippocampus; CB: Cerebellum

In the present study, the SOR generation was suggestively decreased in the supplemented trainees indicating the additive role of physical activity and diet in attenuating the OS. Tuon *et al.*[37] reported that moderate exercise decreases the generation of SOR in the HC region of the brain. The reduced levels of SOR in the HI extract supplemented rats may be attributed to the polyphenols present in the extract which quenches these free radicals.

PC, markers of oxidative protein damage, were reduced maximally in the SW-T(+HI_2) group. Our results indicate that combinations of two interventions were effective in attenuating the oxidative modifications of proteins. Previous studies have also reported that supplementation of plant extracts decreased protein carbonyl levels in the brain.[2,38] Marosi *et al.*[39] also found decreased protein oxidation and reduced OS in HC following the exercise regime.

Lipid peroxidation is the result of free radical-facilitated damage that causes membrane damage and produces a variety of secondary byproducts such as MDA,[40] which is considered as a biomarker of OS. In the present study, supplemented trainees and sedentaries are effective in inhibiting lipid peroxidation in all the three brain regions. The findings are in accordance with the reports of Abhijit *et al.*[36] wherein combined intervention of aerobic exercise and supplementation is effective in ameliorating the lipid peroxidation in the HC region of middle-aged and adult rats.

Thiols are a group of organic compounds possessing a sulfhydryl group (–SH) that actively take part in key functions such as antioxidant defenses, xenobiotic metabolism, and cell cycle regulation.[41] The HC region showed higher thiol levels which suggest a greater OS compared to other regions. The increased thiol levels in the swim trainees and supplemented groups either individually or in combination may be due to the induction and upregulation of the rate-limiting enzyme, gamma-glutamylcysteine synthetase involved in glutathione synthesis. Our findings on increased thiols levels with dietary supplementation and exercise are in line with the reports of Balu *et al.*[42] and Anand *et al.*[24]

CONCLUSION

Our results highlight that the combined interventions of swimming exercise and HI supplementation can improve endogenous antioxidant defense, thereby decreasing the levels of radical generation and damage to biomolecules. The present study revealed that nutrition and exercise attenuate OS in the brain and may corroborate as a potential intervention in improving the antioxidant status of the brain.

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Conflicts of interest

There are no conflicts of interest.

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