Original Article

# Effect of age and physical activity on oxidative stress parameters in experimental rat model

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## Abstract

**Background and Aim:** Studies have suggested that regular physical exercise has beneficial effects on the brain by modulating the oxidative stress. There is little information regarding whether swimming exercise could attenuate oxidative stress in the brain. Therefore, the present study was aimed to investigate the effect of physical activity on the oxidative stress parameters in the cerebral cortex and the hippocampus of the aging rat brain.

**Methods:** Male Wistar albino rats of 4-, 12- and 22-months old age were swim-trained in a rectangular glass tank for 30 min/day, 6 days/week with 3% of load for 4 weeks. We examined the levels of protein carbonyls, superoxide radicals and thiols to assess the oxidative stress with exercise in the aging rat brain.

**Results:** Age-related increase in the protein carbonyls and superoxides with decreased thiols were observed. Hippocampus exhibited higher levels of oxidative stress compared to the cerebral cortex. Training significantly attenuated the oxidative stress as carbonyls and superoxides reduced and thiols were enhanced.

**Conclusion:** The findings of this study suggests that physical exercise reduces the oxidative stress in the cerebral cortex and hippocampus and may provide a therapeutic intervention in attenuating age-related oxidative stress in the brain.

**Key words:** Aging, protein carbonyls, protein thiols, superoxide radical, swimming training

*Received:* 24th July, 2015; *Revised:* 14th September, 2015; *Accepted:* 26th September, 2015

## INTRODUCTION

Aging is defined as the gradual alteration in structure and function that occurs over time, eventually leading to increased probability of nondisease- or nontrauma-related death.[1] Several theories have been offered to understand the phenomenon of aging, among which the free radical theory of aging has gained relatively strong support in this area.[2] Free radicals are generated as an intermediate in biochemical reactions[3] and can participate in side chain reactions resulting in cell damage. Superoxide has a relatively long half-life facilitating diffusion within the cell thereby increasing the number of potential targets. Excessive production of these oxygen-free radicals was demonstrated as a contributing factor in age-related memory and synaptic plasticity dysfunction.[4]

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| Quick Response Code: | Website: www.ijcep.org |
| | DOI: 10.4103/2348-8093.169960 |

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 (PCs) and protein thiols. Protein carbonylation is the most common oxidative modification seen with accelerating age,[5] 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.[6] Thus, oxidative modification of proteins *in vivo* may

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**How to cite this article:** Anand S, Rajashekharaiah V, Tekupalli R. Effect of age and physical activity on oxidative stress parameters in experimental rat model. Int J Clin Exp Physiol 2015;2:185-90.

affect a variety of cellular functions involving proteins, receptors, signal transduction mechanisms, transport systems, and enzymes.

Physical exercise utilizes energy metabolism and synaptic plasticity to promote brain health, upregulating proteins related to cognitive[7] and mitochondrial functions.[8] It has protective effects against several neurological diseases including Parkinson's[9] and Alzheimer's disease.[10] Moreover, it has also been associated with a reduced risk of cognitive impairment and dementia with age.[11] Moderate exercise is said to be associated with enhanced neurogenesis[12] and showed decreased levels of markers of oxidative stress.[13]

Although studies have been reported on the effects of physical exercise on antioxidant status in the brain, the findings are conflicting.[14-16] We chose cerebral cortex (CC) and hippocampus (HC) regions, as they are involved in higher brain functions such as learning, memory, and perception. Based on the above facts, the present study was designed to investigate the effects of swimming exercise on markers of oxidative stress in the CC and HC regions of the aging rat brain.

## MATERIALS AND METHODS

### Chemicals

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), nitroblue tetrazolium (NBT), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were procured from Sigma-Aldrich (St. Louis, MO, USA). All general chemicals and solvents were of reagent grade.

### Animal care

This study was conducted using protocols that were approved by the Institutional Animal Ethics Committee, Bangalore University, Bengaluru, India, and the experiments were carried out in accordance with the guidelines set by Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The study comprised of 30 male albino rats of Wistar strain of 4, 12, and 22 months of age. Initially, rats of 3-months of age were obtained from the Central Animal Facility, Indian Institute of Science, Bengaluru, and maintained until they attained respective ages for experimental studies in our animal facility. The rats were assigned into two groups ($n$ = 5): (i) Sedentary control (SE-C) and (ii) swim-trained (SW-T). Animals were housed 5 per cage in polypropylene fitted steel mesh-bottom cages and were maintained at a temperature of 32 ± 1°C, relative humidity of 77 ± 1%, and under a 12-h of dark and light cycle. All animals had free access to feed (Amrut India Ltd., Bengaluru, India) and tap water *ad libitum*.

### Swimming training protocol

Swimming exercise training was similar to our earlier protocols with minor modifications.[17] Briefly, 4-(young), 12-(middle), and 22-(old) months old rats were made to swim in a rectangular glass tank (77 cm × 38 cm × 39 cm) filled with water to a height of 18, 22, and 26 cm, respectively, at 32 ± 1°C for 5 days/week for 30 days with 3% of their body weight tied to 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 4 weeks with 6 days/week. SE-Cs were restricted to cage activity.

### Sample preparation

After the completion of the training period, the animals were sacrificed immediately under diethyl ether anesthesia. The brain tissue was excised and the CC and HC were separated, weighed, and rinsed several times in phosphate buffer (pH 7.0). The tissues were homogenized in lysis buffer containing HEPES, NaCl, KCl, $MgSO_4$, and 1 mM PMSF. The protein content was estimated by Bradford method using bovine serum albumin as standard.[18]

### Quantitative analysis of protein carbonyls

PCs were measured according to the procedure of Levine *et al*.[19] Briefly, 100 µl of supernatant (tissue extract) was incubated with 0.5 ml of 10 mM dinitrophenylhydrazine (DNPH) in 2M HCl for 60 min in dark at room temperature (RT). Protein was precipitated using 0.5 ml of 20% trichloroacetic acid (TCA) and then centrifuged at 10,000 × $g$ for 3 min at 4°C. The supernatant was discarded and the resultant pellet was washed twice with ethanol: ethyl acetate (1:1 v/v) mixture by centrifuging at 3400 × $g$ for 5 min each to remove excess of DNPH. Precipitated protein was then re-dissolved in 1.5 ml of 6 M Guanidine hydrochloride in 20 mM phosphate buffer (pH - 6.5). Insoluble substances were removed by centrifugation and absorbance of the supernatant was read at 370 nM. An extinction coefficient of 22,000/M/cm was used to determine the PC content and was expressed as nmol/mg protein.

### Measurement of superoxide radicals

Superoxide radicals were measured according to the method of Das *et al*.[20] Briefly, 200 µl of homogenate was incubated with 80 µl of 0.1% NBT in an oscillating water bath for 1 h at 37°C. Termination of the assay and extraction of the reduced NBT were carried out by centrifuging the samples for 10 min at 200 × $g$ then resuspending the pellets in 1 ml of glacial acetic acid. The absorbance was measured at 560 nm and converted to µmoles diformazan using a standard curve generated from NBT diformazan. Final results were expressed as µmoles diformazan/mg tissue.

### Determination of total thiols and nonprotein thiol levels

The thiol groups were determined according to the procedure of Sedlak and Lindsay.[21] 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 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. Color developed in 15 min and the reaction mixture was centrifuged approximately at 3,000 × *g* at RT for 15 min. The absorbance of the supernatants was read in a spectrophotometer at 412 nm.

Aliquots of 250 μl of the homogenates are mixed in 5 ml test tubes with 200 μl distilled 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, and 10 μl 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 with no homogenate. The protein thiols groups were calculated by subtracting the nonprotein thiol from total thiols.

### Statistical analysis of data

All the data were expressed as mean ± standard error and were analyzed within a two-factor analysis of variance between age and groups. When a significant F ratio was found, Duncan's multiple range tests were performed. The statistical analysis was performed using SPSS (Armonk, NY: IBM Corp) 20 software package for Windows. Probability values ($P < 0.05$) were considered significant.

## RESULTS

Table 1 represents the changes in body weight in the young, middle, and old age animals. Irrespective of age, there was a significant decrease ($P < 0.05$) in body weight of the swim trainees. The 4- and 12-month old trained animals showed around 5% reduction in their body weights over the SE-Cs. However, in the 22-month old animals, significant decrease in body weight was observed during the first 3 weeks of training period over their sedentary counterparts.

The PC content, a marker of protein oxidation, increased with age in both the regions as represented in Figure 1a and b. The HC showed significantly ($P < 0.05$) higher carbonyl content compared to the CC in all the three age groups. A significant reduction of 38%, 50%, and 44% was evident in the CC of 4, 12, and 22-month-old trained animals, respectively, in relation to the SE-Cs. Around 50% reduction in the carbonyl content was clear in the HC in all the age groups over their sedentary counterparts.

Figure 2 represents the generation of superoxide radicals in the control and SW-T animals. Superoxide radical generation significantly ($P < 0.05$) increased with age and exhibited region-specific changes. In 4-month-old animals, training significantly reduced the superoxide radical generation by 12% and 43% in CC and HC over the SE-Cs. The superoxide radical generation in the middle-aged animals was decreased by 19% (CC) and 37% (HC) with training. In 22-month-old animals, swim training reduced the superoxide radical content by 28% and 42% (CC and HC) over the sedentary counterparts.

The levels of total thiols, nonprotein thiols, and protein thiols in the control and SW-T animals decreased with age as indicated in Table 2. The CC has a higher content of total, nonprotein, and protein thiols than HC. Training significantly increased ($P < 0.05$) the levels of total thiols

**Table 1:** Changes in the body weight during the training period

| Age (months) | Groups | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 4 | SE-C | 256±3.3 | 265±2.2 | 274±1.8 | 285±1.2* |
| | SW-T | 250±0.9 | 257±1.7# | 263±0.6# | 270±2.4# |
| 12 | SE-C | 356±2.1 | 366±1.9 | 377±1.8 | 386±2.3* |
| | SW-T | 355±2.2 | 360±2.1# | 366±2.2# | 373±1.8# |
| 22 | SE-C | 490±2.3 | 499±3.0 | 505±2.6 | 508±2.0* |
| | SW-T | 488±0.9 | 493±0.9# | 496±0.6# | 499±1.2# |

*Depicts comparison between the SE-C and SW-T is represented using *$P<0.05$, #Depicts in comparison with the 1st week, #$P<0.05$ was considered significant. Values are represented as mean±SE of five animals/group. Statistical analysis was done by two-way ANOVA followed by DMRT. SE-C: Sedentary control, SW-T: Swim trained, SE: Standard error, ANOVA: Analysis of variance, DMRT: Duncan's multiple range tests

**Table 2:** The levels of TT, NPT, and PT in the cerebral cortex (A) and hippocampus (B) as a function of age and exercise

| Parameters | Groups | 4 months | 12 months | 22 months |
|---|---|---|---|---|
| | | **Cerebral cortex (A)** | | |
| TT | SE-C | 130±0.1 | 124±0.1 | 100±0.2* |
| | SW-T | 152±0.1 | 140±0.5# | 126±0.5# |
| NPT | SE-C | 80±0.2 | 75±0.3 | 60±0.4* |
| | SW-T | 92±0.3 | 85±0.2# | 75±0.4# |
| PT | SE-C | 50±0.2 | 49±0.4 | 40±0.2* |
| | SW-T | 60±0.1 | 55±0.6# | 51±0.1# |
| | | **Hippocampus (B)** | | |
| TT | SE-C | 120±0.1 | 110±0.2 | 90±0.2* |
| | SW-T | 141±0.2 | 130±0.3# | 112±0.4# |
| NPT | SE-C | 75±0.3 | 64±0.2 | 53±0.3* |
| | SW-T | 87±0.4 | 76±0.1# | 67±0.5# |
| PT | SE-C | 45±0.3 | 46±0.4 | 37±0.1* |
| | SW-T | 54±0.7 | 54±0.4# | 45±0.8# |

*Depicts comparison between the SE-C and SW-T is represented using *$P<0.05$, #Depicts in comparison with the 4 months, #$P<0.05$ was considered significant. Data are expressed as mean±SE of five animals/group. Units: nmol/mg protein. Statistical analysis was done by two-way ANOVA followed by DMRT. SE-C: Sedentary control, SW-T: Swim trained, TT: Total thiols, NPT: Nonprotein thiols, PT: Protein thiols, SE: Standard error, ANOVA: Analysis of variance, DMRT: Duncan's multiple range tests

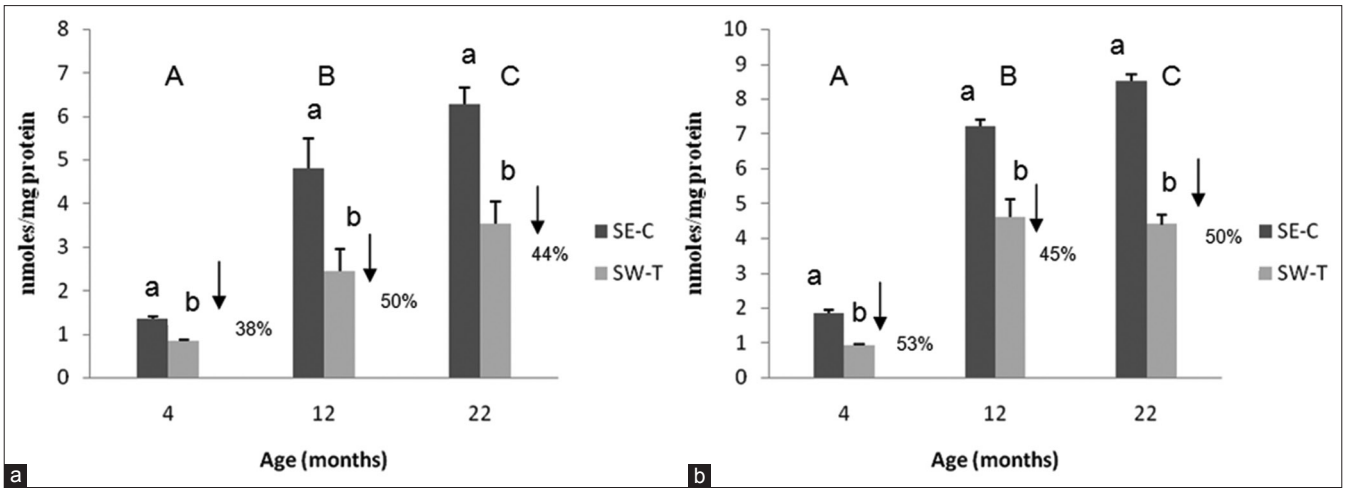


**Figure 1:** Protein carbonyl content in the cerebral cortex (a) and hippocampus (b) of sedentary control and swim trained rats as a function of age and exercise. ↓ represent percentage decrease in protein carbonyl levels in swim trained rats with respect to control. Values are expressed as mean ± standard error of 5 animals/group. Significance between age groups means was analyzed by two-way analysis of variance followed by Duncan's multiple range tests and is represented in upper case and within groups in lower case. Those not sharing the same letters are significantly different at $P < 0.05$. SE-C: Sedentary control; SW-T: Swim trained

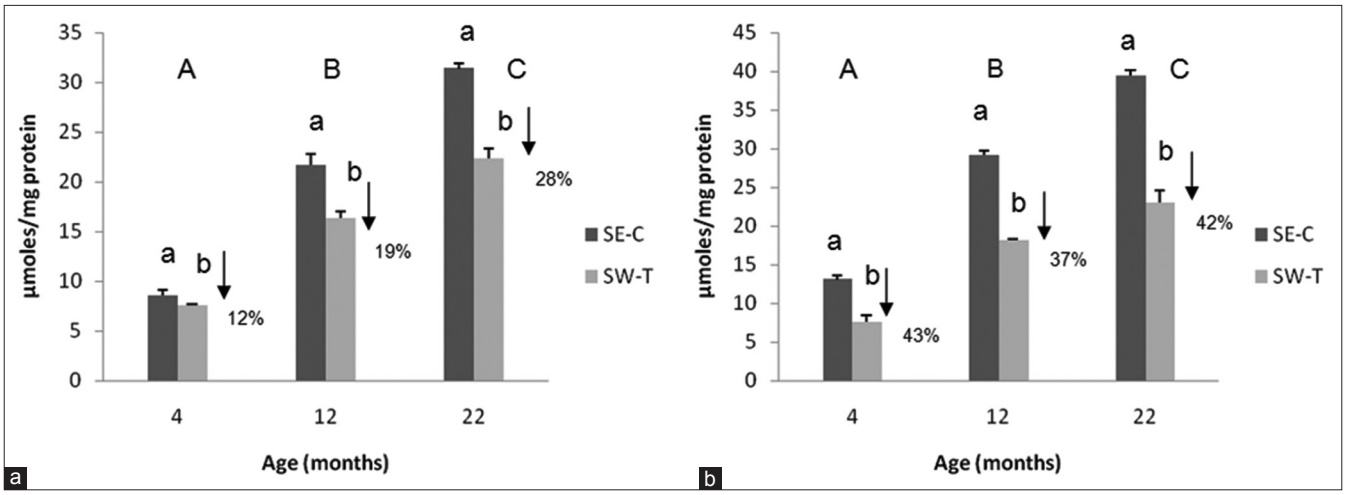


**Figure 2:** Superoxide radical levels in the cerebral cortex (a) and hippocampus (b) of sedentary control and swim trained rats as a function of age and exercise. ↓ represent percentage decrease in superoxide radical levels in swim trained rats with respect to control. Values are expressed as mean ± standard error of 5 animals/group. Significance between age groups means was analyzed by two-way analysis of variance followed by Duncan's multiple range tests and is represented in upper case and within groups in lower case. Those not sharing the same letters are significantly different at $P < 0.05$. SE-C: Sedentary control; SW-T: Swim trained

by 26% (CC) and 27% (HC) in the old age animals when compared to their sedentary counterparts. An increase of nonprotein thiol levels by 25% in CC and 27% in HC were evident in SW-T animals of old animals over the SE-C. The protein thiol levels elevated in the old age animals by 28% and 22% in CC and HC with respect to their SE-Cs.

## DISCUSSION

Exercise has shown to induce several beneficial effects to the central nervous system of humans[22,23] and animals[24,25] such as improved learning, memory and plasticity,[26,27] promoting neuronal activation,[28,29] and increased neurogenesis.[25] Studies by Radak *et al*. and Somani *et al*. revealed conflicting results on the effects of physical exercise on oxidative damage in the brain.[14,16,30] The purpose of the present study was to determine whether swimming exercise can attenuate the age-related oxidative stress in the brain. We selected swimming as a model for exercise performance, because swimming appears to be a natural behavior of rodents.[31] It is known that swimming training has been used as a suitable method of endurance training because of several benefits derived out of it over treadmill running, a few of these include the possible differences in the sympathoadrenal function between swimming and running, less mechanical stress and injury during swimming due to buoyancy and reduced effects

of gravity, as well as better redistribution of blood flow among tissues without significant variations in cardiac output, and heart rate that in turn minimizes the magnitude of injury caused due to the generation of ROS.

Our study demonstrated a significant decrease in the body weight ($P < 0.05$) of the swim trainees in relation to their sedentary counterparts in all the age groups which indicates the beneficial effects of swim exercise in reducing the body weight. Previous studies from our laboratory have also reported a significant decrease in body weight when animals were SW-T for 30 min with 3% load.[32]

The central nervous system is particularly vulnerable to oxidative damage due to high utilization of inspired oxygen, large amount of easily oxidizable polyunsaturated fatty acids, abundance of redox-active transition metal ions, and relative deficiency of antioxidant defense systems.[6] The CC and HC regions of the brain are widely believed to be critically involved in memory encoding and development of memory and related to locomotor responses. Hence, the increase in oxidative damage may be particularly critical to these regions of brain.[33] Therefore, we made an attempt to evaluate the antioxidant status of CC and HC regions of the aging rat brain in response to swimming exercise.

Protein carbonylation is one of the most common oxidative modifications seen with accelerating age[5] and can be assessed by measuring the carbonyl levels. In the present study, the PCs were higher in HC in comparison with CC in all the age groups. The results are in agreement with the findings of Marosi *et al*.[34] This is due to HC being highly vulnerable to oxidative damage during aging due to the reduced capacity of neurons to maintain redox homeostasis.[35] Training significantly reduced the carbonyl levels in different age groups thereby suggesting the positive role of physical exercise in combating oxidative stress. Studies by Ogonovszky *et al*.[36] also showed the reduction in carbonyl content with swimming exercise.

Superoxide, one of the major free radical, having a relatively long half-life significantly increased with age. Our results demonstrated a significant reduction in superoxide radical generation with training in different age groups. HC showed a higher radical generation compared to CC, as this region is more prone to oxidative stress. Previous studies by Balu *et al*. also reported higher radical generation in the HC of aged rats compared to the young rats.[6] This suggests the role of physical exercise in attenuating the generation of free radicals by probably up-regulating the superoxide dismutase activity in the brain.

Thiol compounds are said to be the natural reservoir of reductive capacity of a cell. The most vital of the multifaceted roles played by thiols *in vivo* is their function as entities of the intracellular and extracellular redox buffer. The levels of these molecules decrease with age. The decrease in thiols in the brain leads to imbalance between different redox forms of thiols that in turn lead to impaired protection of protein sulfhydryl groups upon irreversible oxidation. The present study demonstrated a significant increase ($P < 0.05$) in the levels of total thiols, nonprotein thiols, and protein thiols with training in the CC and HC regions of the brain in all the age groups. The up-regulation of the thiols with exercise indicates the beneficial role of physical exercise in maintaining the redox signaling. Previous studies by Cechetti *et al*. and Jolitha *et al*. also showed increased cellular thiols in different regions of the brain when the rats were subjected to treadmill and swim exercise.[37,38]

### Limitations of the study

In the present study, the control rats were caged, instead these rats could have been made to be in contact in water of water for 30 min/day for 4 weeks. Then the stress of being in water environment would have been a proper basal value of oxidative stress. Further to assess the role of physical activity on oxidative stress, basal parameters could have been studied in a group of rats before administering physical activity as control to study the effect of physical activity (pre- and post-) on oxidative stress.

## CONCLUSION

The present study demonstrated the role of physical exercise in attenuating the levels of oxidative stress markers in the CC and HC of aging rat brain. Therefore, physical exercise may be helpful in overcoming age-related oxidative stress in the different regions of the brain and may act as an adjuvant in treating neurodegenerative disorders.

### Acknowledgments

We thank Mrs. Sowbhagya R, Mrs. Anupama SK and Ms. Bhagyalakshmi D for technical assistance.

### Financial support and sponsorship

This study was supported by the financial assistance from the University Grants Commission, New Delhi, India (F.No: 34–267).

### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. McCord MC, Aizenman E. The role of intracellular zinc release in aging, oxidative stress, and Alzheimer's disease.

Front Aging Neurosci 2014;6:77.
2. Perluigi M, Swomley AM, Butterfield DA. Redox proteomics and the dynamic molecular landscape of the aging brain. Ageing Res Rev 2014;13:75-89.
3. Halliwell B, Gutteridge JM. The definition and measurement of antioxidants in biological systems. Free Radic Biol Med 1995;18:125-6.
4. Massaad CA, Klann E. Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid Redox Signal 2011;14:2013-54.
5. Butterfield DA, Stadtman ER. Protein oxidation processes in aging brain. Adv Cell Aging Gerontol 1997;2:161-91.
6. Balu M, Sangeetha P, Murali G, Panneerselvam C. Age-related oxidative protein damages in central nervous system of rats: Modulatory role of grape seed extract. Int J Dev Neurosci 2005;23:501-7.
7. Ding Q, Vaynman S, Souda P, Whitelegge JP, Gomez-Pinilla F. Exercise affects energy metabolism and neural plasticity-related proteins in the hippocampus as revealed by proteomic analysis. Eur J Neurosci 2006;24:1265-76.
8. Kirchner L, Chen WQ, Afjehi-Sadat L, Viidik A, Skalicky M, Höger H, *et al*. Hippocampal metabolic proteins are modulated in voluntary and treadmill exercise rats. Exp Neurol 2008;212:145-51.
9. Smith AD, Zigmond MJ. Can the brain be protected through exercise? Lessons from an animal model of parkinsonism. Exp Neurol 2003;184:31-9.
10. Mirochnic S, Wolf S, Staufenbiel M, Kempermann G. Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. Hippocampus 2009;19:1008-18.
11. Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001;58:498-504.
12. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 2005;25:8680-5.
13. Foster KA, Galeffi F, Gerich FJ, Turner DA, Müller M. Optical and pharmacological tools to investigate the role of mitochondria during oxidative stress and neurodegeneration. Prog Neurobiol 2006;79:136-71.
14. Radak Z, Sasvari M, Nyakas C, Kaneko T, Tahara S, Ohno H, *et al*. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. Neurochem Int 2001;39:33-8.
15. Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, *et al*. Chronically and acutely exercised rats: Biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol (1985) 2000;89:21-8.
16. Somani SM, Ravi R, Rybak LP. Effect of exercise training on antioxidant system in brain regions of rat. Pharmacol Biochem Behav 1995;50:635-9.
17. Anand S, Devi SA, Ravikiran T. Differential expression of the cerebral cortex proteome in physically trained adult rats. Brain Res Bull 2014;104:88-91.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
19. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994;233:346-57.
20. Das UN, Padma M, Sagar PS, Ramesh G, Koratkar R. Stimulation of free radical generation in human leukocytes by various agents including tumor necrosis factor is a calmodulin dependent process. Biochem Biophys Res Commun 1990;167:1030-6.
21. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
22. Dustman RE, Emmerson RY, Ruhling RO, Shearer DE, Steinhaus LA, Johnson SC, *et al*. Age and fitness effects on EEG, ERPs, visual sensitivity, and cognition. Neurobiol Aging 1990;11:193-200.
23. Lupinacci NS, Rikli RE, Jones CJ, Ross D. Age and physical activity effects on reaction time and digit symbol substitution performance in cognitively active adults. Res Q Exerc Sport 1993;64:144-50.
24. Real CC, Ferreira AF, Hernandes MS, Britto LR, Pires RS. Exercise-induced plasticity of AMPA-type glutamate receptor subunits in the rat brain. Brain Res 2010;1363:63-71.
25. Ferreira AF, Real CC, Rodrigues AC, Alves AS, Britto LR. Short-term, moderate exercise is capable of inducing structural, BDNF-independent hippocampal plasticity. Brain Res 2011;1425:111-22.
26. Lambert TJ, Fernandez SM, Frick KM. Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. Neurobiol Learn Mem 2005;83:206-16.
27. Vaynman SS, Ying Z, Yin D, Gomez-Pinilla F. Exercise differentially regulates synaptic proteins associated to the function of BDNF. Brain Res 2006;1070:124-30.
28. Holschneider DP, Yang J, Guo Y, Maarek JM. Reorganization of functional brain maps after exercise training: Importance of cerebellar-thalamic-cortical pathway. Brain Res 2007;1184:96-107.
29. Lewis DA, González-Burgos G. Neuroplasticity of neocortical circuits in schizophrenia. Neuropsychopharmacology 2008;33:141-65.
30. Radak Z, Asano K, Inoue M, Kizaki T, Ohishi S, Suzuki K, *et al*. Acute bout of exercise does not alter the antioxidant enzyme status and lipid peroxidation in rat hippocampus and cerebellum. Pathophysiology 1995;2:243-5.
31. Lima FD, Stamm DN, Della-Pace ID, Dobrachinski F, de Carvalho NR, Royes LF, *et al*. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. PLoS One 2013;8:e55668.
32. Devi SA, Kiran TR. Regional responses in antioxidant system to exercise training and dietary Vitamin E in aging rat brain. Neurobiol Aging 2004;25:501-8.
33. Parihar MS, Chaudhary M, Shetty R, Hemnani T. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: Prevention by extracts of *Withania somnifera* and *Aloe vera*. J Clin Neurosci 2004;11:397-402.
34. Marosi K, Bori Z, Hart N, Sárga L, Koltai E, Radák Z, *et al*. Long-term exercise treatment reduces oxidative stress in the hippocampus of aging rats. Neuroscience 2012;226:21-8.
35. Serrano F, Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res Rev 2004;3:431-43.
36. Ogonovszky H, Berkes I, Kumagai S, Kaneko T, Tahara S, Goto S, *et al*. The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. Neurochem Int 2005;46:635-40.
37. Cechetti F, Worm PV, Elsner VR, Bertoldi K, Sanches E, Ben J, *et al*. Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat. Neurobiol Learn Mem 2012;97:90-6.
38. Jolitha AB, Subramanyam MV, Asha Devi S. 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 2006;41:753-63.