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Antioxidant action of grape seed polyphenols and aerobic exercise in improving neuronal number in the hippocampus is associated with decrease in lipid peroxidation and hydrogen peroxide in adult and middle-aged rats

S. Abhijit[a], Sunil Jamuna Tripathi[b], Bhagya V.[b], Shankaranarayana Rao B.S.[b], Muthangi V. Subramanyam[c], S. Asha Devi[a,*]

[a] Laboratory of Gerontology, Department of Zoology, Bangalore 560 056, Karnataka, India
[b] Department of Neurophysiology, National Institute of Mental Health and Neuro Sciences, Bengaluru 560 029, Karnataka, India
[c] Department of Life Science, Bangalore University, Bangalore 560 056, Karnataka, India

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ABSTRACT

The present study explored the effects of swimming training and grape seed proanthocyanidin extract (GSPE) on neuronal survival in the hippocampus (HC) of middle-aged rats along with oxidative stress (OS) parameters. Further, the bioavailability of the GSPE, catechin, epicatechin and gallic acid were measured in the HC and plasma. Male *Wistar* rats were grouped into: sedentary control, SE-C; swimming trained, SW-T; SE-C, supplemented sedentary, SE-C(PA) and swimming trainees, SW-T(PA). The supplement was a daily dose of 400 mg GSPE/kg body weight. Swimming training lasted for 2 h/day and for 14 weeks. Glutathione level was increased in response to single and combined interventions in the middle-aged rats. Adult trainees showed increased glutathione peroxidase activity unlike middle-aged wherein increase was seen in SE-C(PA) alone. Lowered catalase activity with age in the HC increased in response to the combined interventions although single interventions were also effective. HC from both ages showed decrease in lipid peroxidation and hydrogen peroxide levels in response to the interventions. GSPE constituents were seen in the HC of swimming trained middle-aged and adult rats. The study suggests that combined intervention is effective in decreasing LPO and H_2O_2 generation in the HC. Further, the neuronal numbers and planimetric volumes of CA1 pyramidal layer was significantly reduced in middle-aged rats compared to adults. Interestingly, both interventions enhanced the numbers and volumes in adult and middle-aged rats. Thus, age-associated decrease in CA1 neurons could be restored by both the interventions. The results of the present study will help in developing effective therapies for age-associated degenerative changes and cognitive deficits.

1. Introduction

Brain aging is primarily associated with an imbalance of the pro- and antioxidant factors that lead to neuronal damage and decline in cognitive functions. The most adversely affected region, however, is the hippocampus (HC) (Burke and Barnes, 2010; Floyd and Hensley, 2002). Brain aging is also associated with progressive decline in antioxidant enzymes (Jolitha et al., 2006; Zhu et al., 2006). Glutathione (GSH) forms the primary line of defense against hydrogen peroxide (H_2O_2)-mediated oxidative stress (OS) by detoxifying activity of glutathione peroxidase (GPx) and catalase (CAT). Additionally, the defense system scavenges superoxide (O_2^-), hydroxyl (OH) and oxidant peroxynitrites ($ONOO^-$) (Dringen et al., 2000). Among all the cortico-temporal areas of brain, hippocampus is most vulnerable to the age –related changes in mitochondrial activity and OS (Navarro et al., 2008). Further, the neurons in the subfield CA1 of the hippocampus is more vulnerable to OS than those of the CA3 (Wang et al., 2005).

Studies have shown the possibilities of enhancing the antioxidant defense of the aging brain through grape seed polyphenol supplements and in turn improving the spatial memory (Asha Devi et al., 2011, 2006). Grape seed proanthocyanidin extract (GSPE) is a natural antioxidant containing the major phenolic acid, gallic acid (GA, 3, 4, 5-trihydroxybenzoic acid) and polyphenols, (+) catechin (C), (−) epicatechin (EC) and procyanidin dimers B1, B2, C1 (Nakamura et al., 2003). Gallic acid (GA) is a potent antioxidant by virtue of its efficient hydrogen donor capability whereas catechin (C) and epicatechin (EC) are efficient radical scavengers (Muzolf-Panek et al., 2012). Apart from being efficient free radical scavengers, C and EC are also potent

* Corresponding author.
E-mail address: sambeashadevi@bub.ernet.in (S. Asha Devi).

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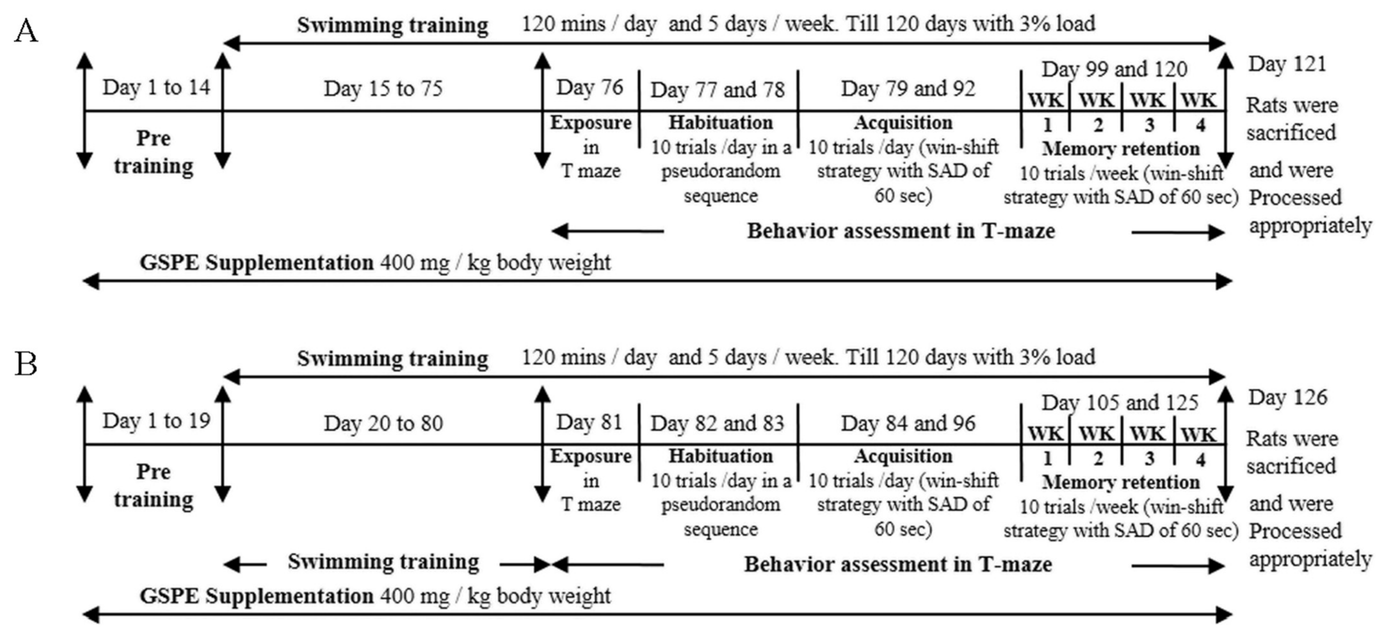

Fig. 1. Representative Time line of exercise paradigm of adult (A) and middle-aged (B) rats.

transition metal chelators, especially for Cu (II) and Fe (II) (Karamać, 2009; Mandel et al., 2006) and thus their presence may make the brain more resistant to free radical-mediated oxidative stress. Few studies have shown that polyphenols, especially flavanols (Milbury and Kalt, 2010; Prasain et al., 2009; Abd El Mohsen et al., 2002) and resveratrol (Juan et al., 2010) are deposited in the brain regions following oral supplementations. Further, studies related to regular exercise have shown the protective effect of exercise training in terms of an adaptive response to OS in the brain (Radak et al., 2014; Lafay et al., 2009; Aguiar and Pinho, 2007) with aging (Asha Devi and Ravi Kiran, 2004; Sen and Packer, 2000). However, very few reports exist with respect to the possible benefits derived by combining exercise training of aerobic nature with GSPE to derive synergistic effects, if any, in the hippocampus.

Accordingly, the present study on hippocampus was based the following hypotheses: (1) exercise and GSPE, may be effective in curtailing lipid peroxidation (LPO) and hydrogen peroxide (H_2O_2) generation; (2) the effect of the two interventions may be a function of bioavailability of unmetabolized polyphenols in the hippocampus; (3) The stereology of the aged hippocampus may be related to LPO and H_2O_2 levels that are indicative of cellular metabolism. The hypotheses were examined through a comprehensive determination of the antioxidant, GSH and antioxidant enzymes, markers of cellular oxidative stress, the bioavailability of constituents of GSPE in the plasma and hippocampus, and finally, the neuronal number and volume of the CA1 subfield of the hippocampus.

2. Materials and methods

2.1. Animals

All procedures involving animals were approved by the Institutional Animal Ethics Committee (IAEC) of Bangalore University, Bangalore, India and complied with the guidelines of the Control for the Purpose and Supervision of Experiments on Animals (CPCSEA).

Wistar male rats of 2 months of age were obtained from the Central Animal Facility of the Indian Institute of Science (Bangalore) and were maintained until they reached 4- (adult) and 18-(middle-aged) months of age. They were maintained at 22 ± 1 °C with 12-h light/dark cycle. Rats were fed with standard laboratory chow (Amrut Feeds, Bangalore) and tap water *ad libitum.*

2.2. Experimental grouping and supplementation

A total of 72 animals, 36 animals from each age group, were divided randomly into four sub-groups: (1) sedentary controls, SE-C; (2) sedentary supplemented with GSPE, SE-C (PA); (3) swimming trained (SW-T); and (4) swimming trainees supplemented with GSPE, SW-T (PA). Control and swimming trainees remained on normal diet with a supplement of distilled water. Supplementees received an oral dose of 400 mg/kg body weight/day of GSPE for a total period of 16 weeks. Gravinol (Kikkoman Co. Ltd.; Noda, Japan) was a generous gift from Prof. Naokata Ishii from the University of Tokyo, Komaba, Tokyo, Japan. Gravinol is a natural substance extracted from grape seeds with ethanol and water as eluents and then purified and condensed. It contains 96% proanthocyanidin and other minor components and is referred as grape seed proanthocyanidin extract (GSPE).

2.3. Swimming training

Each day the rats were transferred to the exercise training room and allowed to swim in a circular tank of 51 cm diameter, with water at a depth of 21 cm for the adults and 29 cm for the middle-aged. Swimming training was initiated with pre-training the animals to swim with a load of 3% of their body weight tied to their tails. During pre-training, the duration was increased progressively from 5 min till they swam for 2 h and thereafter continued for 16 weeks as the training period. The pre-training period, however, lasted for 21 days for the middle-aged animals against 14 days for the adults as shown in Fig.1. Animals were dried after the swim exercise and placed back into their housing cages. In addition, during the training sessions sedentary adult and middle-aged animals were transferred to the training room but remained in their home cages. At the end of the experiment, rats were sacrificed to estimate the bioavailability of gallic acid, catechin and epicatechin in the HC.

2.4. Preparation of plasma and tissue samples

2 h following the last GSPE supplementation, animals were anesthetized with 1.5% halothane (Piramal Healthcare, India). 1 ml blood was collected in EDTA-coated tubes and centrifuged at 1000 xg for 10 min at 4 °C to obtain the plasma (Kubota, Model 6200, Japan). Following this, animals were transcardially perfused with ice-cold