National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Chronic administration of vitamin C increases cognitive function in chronic stress-induced rats

Sangeetha A[1], Surapaneni Krishna Mohan[2], Kumaresan M[3]

[1]Department of Physiology, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India, [2]Department of Biochemistry, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India, [3]Department of Anatomy, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India

Correspondence to: Surapaneni Krishna Mohan, E-mail: krishnamohan.surapaneni@gmail.com

Received: June 09, 2017; **Accepted:** June 23, 2017

ABSTRACT

Background: Stress and increase in free radicals levels are known to alter cognition, learning, memory, and emotional responses. There is a marked impairment of hippocampus-dependent memory and suppression of long-term potentiation in the CA1 region of the hippocampus during stress. **Aim and Objective:** The aim of this study is to evaluate the hypothesized neuroprotective effect of oral antioxidants and piracetam in chronic restraint stress-induced rats. **Materials and Methods:** Healthy Wistar rats were divided into 5 groups ($n = 6$) each. Control group received neither stress nor oral antioxidant. Stress group received chronic restraint stress for 6 h per day for 21 days. Three experimental groups were administered vitamin C (100 mg/kg), beta-carotene (7 mg/kg), and caffeine (8 mg/kg), respectively. Evaluation parameters were measurement of serum nerve growth factor (NGF) using ELISA method. The oxidative stress markers, glutathione peroxidase (GPx), glutathione reductase, and malondialdehyde were measured. Histological analysis of CA1 region of the hippocampus was done to evaluate the structural changes of pyramidal neurons. **Results:** Vitamin C caused statistically significant ($P < 0.001$) increase in serum NGF. The results revealed that vitamin C caused statistically significant ($P < 0.05$) increase in antioxidant enzymes GPx, glutathione reductase. Vitamin C caused increase in neuronal cell size and volume in CA1 pyramidal layer of hippocampus. **Conclusion:** The findings of study are suggestive of neuroprotection, offered by administration of vitamin C compared to other antioxidants against chronic restraint stress-induced rats. These naturally available dietary vitamins might serve as an adjuvant therapy to avoid progression of brain damage during stress.

KEY WORDS: Nerve Growth Factor; Vitamin C; Beta-Carotene; Caffeine; Pyramidal Neurons

INTRODUCTION

The endogenous proteins that stimulate and control neurogenesis are collectively termed as neurotrophic factors. The neurotrophic factors are generally divided into three subgroups: Neurotrophins, glial cell line-derived neurotrophic factor, and the neuropoietic cytokines. The neurotrophin family includes brain-derived neurotrophic factor (BDNF), neurotrophin-3, neurotrophin-4, and nerve growth factor.[1]

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0621123062017	

BDNF and NGF are the most abundant and widely distributed neurotrophins in the brain. It plays an important role in various aspects of neuronal plasticity, such as neurogenesis, learning and memory, long-term potentiation (LTP), and mood changes.

Neurotrophins are synthesized as pre-proneurotrophin precursors undergo post-translational modifications to become mature form. In the central nervous system, the main

target for NGF is cortex and hippocampus. The medial neurons of nucleus basalis magnocellularis in the cortex and medial septum of hippocampus where the highest amount of NGF are found.[2,3] NGF binds to TrkA receptors. It promotes growth and survival of neurons and it is important for the survival of forebrain cholinergic neurons project to the hippocampus, which are involved in cognitive function. The hippocampus plays an important role in spatial memory, which is essential to learn and remember behaviorally relevant places such as the location of food. The hippocampus is implicated in all stages of processing of spatial memory, including acquisition, recall, and consolidation.[4-6]

Stress may be described as any challenge, either internal or external, that has the potential to disturb the maintenance of homeostasis. Particularly, single or repeated restraint stress application can make modification in neurotrophin mRNA expression in hippocampus and hypothalamus.[7,8] There is a marked impairment of hippocampus-dependent memory and suppression of LTP in the CA1 region of the hippocampus during chronic restraint stress.

Diet plays a very significant role in maintaining and improving overall health and it is no different for mental health as well. Dietary antioxidants have been shown to protect neurons against a variety of experimental neurodegenerative conditions. Several natural beverages, in particular herbal teas, have potential against a variety of oxidative stress-induced neurodegenerative diseases. Foods rich in vitamins C, E, A, and K work as effective natural antioxidants that help improve memory and concentration. Several studies have shown antioxidant vitamins appear to enhance learning and memory through increase neuron survival and synaptic response. However, none of the studies have proved which antioxidant would a better one to improve cognitive function.

Objective

The purpose of this study is to evaluate and to compare the protective effect of various antioxidants in the development of cognitive function and concludes which among these would be a better choice to improve cognitive function during oxidative stress.

MATERIALS AND METHODS

Animal Model

The study was conducted at Center of Laboratory Animal for Research - Department of Research and Development, Saveetha University, Chennai. Before starting with the experiment, the experimental protocol was subjected to scrutiny by the Institutional Ethical Committee for Experimental Clearance with the clearance number SU/BRULAC/RD/002/2015. For this experiment, 42 female Wistar albino rats weighing 150-220 g were used. The care and maintenance of the animal were provided as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. All the rats were housed in a polypropylene cage under standard laboratory conditions with food and water provided *ad libitum*. They were housed under the same standard conditions: 21 ± 11°C, 40-50% humidity, and a 12 h light-dark cycle (light on 07:00 am).

Experimental Design

For this study, totally 30 rats were used and they were divided into five groups. All the rats were weighed every week and the oral antioxidants were given according to the body weight. Distilled water is used as a vehicle. Group I: (n = 6) control - administered distilled water for 21 days. All the rats underwent chronic restraint stress except normal control group. Group II: (n = 6) stress group - Exposed to restraint stress for 6 h per day for 21 days. Oral antioxidants were administered once a day for 21 days before restraint stress exposure. Group III: (n = 6) vitamin C (100 mg/kg) and Group IV: (n = 6) beta-carotene (7 mg/kg) were administered. Group V: (n = 6) caffeine (8 mg/kg).

Restraint Stress Protocol

All the rats underwent chronic restraint stress except normal control group. Rats were kept inside the restrainer for 6 h per day for 21 consecutive days. All the animals were placed individually inside the restrainer. Material used to produce chronic restraint stress is made up of stainless steel with length 15 cm × breadth 7 cm × width 9 cm. The starting time and ending time were kept constant. The temperature was maintained at 18 ± 2°C during stress period that was measured using thermometer under observation. During restraint period, all the rats had free access to water and food. After being restrained for 6 h, the animals were returned to their home cage and given food and water *ad libitum*. The food consumption and body weight of the mice were monitored daily. Unstressed controls remained undisturbed in the separate ventilated cages.

Measurement of Serum Neurotrophic Factor

After 16 h of last restraint session, blood is withdrawn and biochemical analysis was done. Serum NGF was measured using ELISA kit, Boster Biological Technology Co., Ltd., Pleasanton, CA.

Assay of NGF

Boster's rat NGF ELISA kit was based on standard sandwich enzyme-linked immunosorbent assay technology. A monoclonal antibody from mouse specific for NGF has been precoated onto 96-well plates. Standards and test samples are added to the wells; a biotinylated detection polyclonal antibody from goat specific for NGF is added subsequently

and then followed by washing with Tris-buffered saline (TBS) buffer. Avidin-biotin-peroxidase complex was added, and unbound conjugates were washed away with phosphate-buffered saline or TBS buffer. Horseradish peroxidase (HRP) substrate 3,3',5,5'-Tetramethylbenzidine (TMB) was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the rat NGF amount of sample captured in plate.

Estimation of Erythrocyte Glutathione Peroxidase Activity (GPx)

The GPx activity in erythrocytes was estimated by the method of Wendel. The reaction mixture in a total volume of 5.6 ml contained 2 ml of phosphate buffer, 0.5 ml of EDTA, 0.5 ml of sodium azide, 0.1 ml of erythrocyte lysate, 0.5 ml of glutathione (4 mm), and 1.0 ml of H_2O_2 (1.25 mm). After centrifugation, the supernatants were assayed for glutathione content using DTNB. The activity of GPx is expressed as U/g hemoglobin of erythrocyte lysate.

Histological Study of Hippocampal Region

Standard procedures were followed to dissect hippocampus and it was processed for paraffin embedding for microscopic section. Routine stain combination of hematoxylin and eosin, commonly referred as H and E, is used to study the details. Analysis of neuron cell bodies in the pyramidal cell layer of CA-1 region of hippocampus was performed. The slides were viewed under a light microscope and photomicrographs were taken at a total (×10 and ×40).

Statistical Analysis

All behavioral data were analyzed using one-way analysis of variance (ANOVA) followed by *post-hoc* tests (Turkey, HSD) carried out to determine the significant level between groups. Results were expressed as mean ± standard error mean (SEM), $P < 0.001$ was taken as accepted level of significant difference from control.

RESULTS

Serum NGF Levels

The values of serum NGF among the various study groups, expressed as mean ± SEM, are shown in Figure 1.

Applying one-way analysis of variance, the difference between the mean NGF levels for study groups was found to be statistically significant, with $P < 0.01$, $F = 3.215$. Applying Tukey's post-test, the findings are summarized in Table 1.

The mean serum NGF level, as compared to the stress group (Group II), was higher in the vitamin C (Group III), study group, and this difference was significant on statistical analysis ($P < 0.05$).

Table 1: Serum NGF levels: Tukey's post-test

Tukey's post-test	*P* value
Group II versus Group III	<0.05

NGF: Nerve growth factor

Glutathione Peroxidase

The reaction mixture containing 0.4 ml buffer, 0.1 ml sodium azide, 0.2 ml reduced glutathione, required amount of enzyme, 0.1 ml hydrogen peroxide and water was taken to a final incubation volume 2.0 ml. The tubes were incubated at 30° C for 10 minutes. The reaction was terminated by the addition of 0.5 ml TCA. To determine the residual GSH content, the supernatant was removed by centrifugation and added to 2.0 ml of precipitating reagent and 1.0 ml of DTNB reagent. The colour was read at 412 nm. A blank was prepared with only sodium dihydrogen phosphate and 1.0 ml of DTNB reagent. Suitable aliquots of the standard were taken and treated in the same manner.

Glutathione Reductase

The reaction mixture containing 2.2 ml of phosphate buffer, 0.1 ml of EDTA, GSSG and NADPH and this was made up to 3 ml with water. The change in absorbance was monitored after adding suitably diluted enzyme sample at 340 nm for 3 min at 30 seconds intervals in a Shimadzu UV spectrophotometer.

Pyramidal Neurons in CA1 Region of Hippocampus

In this study, changes in hippocampal region were examined histologically to establish the effect of stress and protective role of drugs treatment.

DISCUSSION

The present study showed a decrease in serum NGF after 21 days of chronic restraint stress. These results suggest that increased oxidative stress is associated with lower NGF levels. Interestingly, NGF has slightly increased in caffeine group. Vitamin C group has shown to decrease the neuronal degeneration caused due to stress when compared to other antioxidants. In the present study, rats who received both stress and vitamin C (100 mg/kg dose) have shown beneficiary effect, as its value showed more than to the level of control group. In the present study, beta-carotene administration showed decreased neuroprotection against chronic stress. Therefore, this study clearly explains that administration of beta-carotene alone cannot produce neuroprotection against stress. Figures 3 and 4 also showed increased neuronal survival in hippocampal region

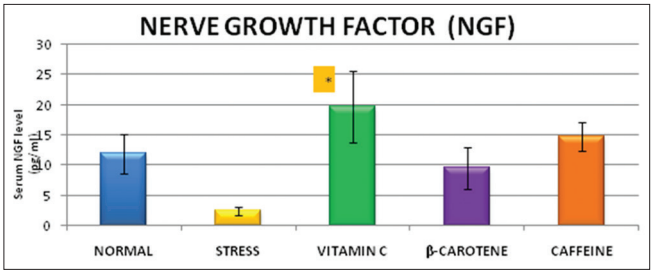

Figure 1: Serum nerve growth factor in rats. Values are shown in mean ± standard error (n = 6). (*): Statistically significant from stress ($P < 0.05$)

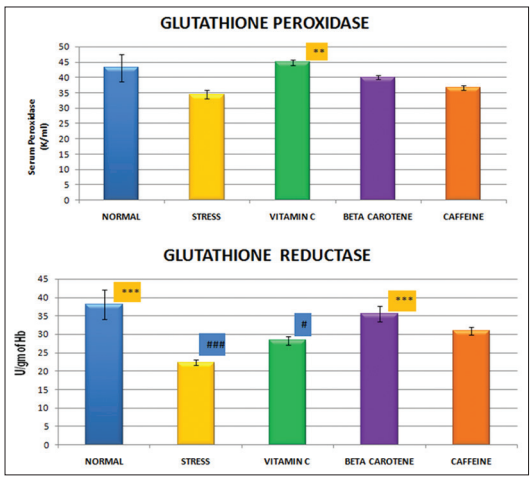

Figure 2: Glutathione peroxidase and glutathione reductase levels in rats. Values are shown in mean ± standard error (n = 6). #Statistically significant from normal ($P < 0.05$). ###Highly statistically significant from normal ($P < 0.001$), **statistically significant from stress ($P < 0.01$), ***highly statistically significant from stress ($P < 0.001$)

of vitamin C group. Adding to this, Figure 2 showed increased glutathione reductase and glutathione peroxidase activity.

Several mechanisms by which oxidative stress could decrease neurotrophins have been suggested, including decreased CREB, increased NF-kB DNA-binding activity,[9,10] or energy depletion[11] According to the literature, decreased serum neurotrophins cause decline in cognitive function. Dietary antioxidants, such as vitamin C, beta-carotene, help to limit the excessive generation of reactive oxygen species. Vitamin C at 100 mg/kg dose in stressed rats has enhanced NGF level when compared to rats who received only stress. These results prove the neuroprotective effect of vitamin C in enhancing the NGF expression.

Considering previous findings and current results, vitamin C found to be a most powerful antioxidant which can combat stress. Vitamin C not only fights against stress but also enhances cognitive function. The probable mechanism could be due to presence of sodium-dependent vitamin C transporter 2 (SVCT 2), a specific transporter for vitamin C in brain particularly hippocampus. A sufficient local buildup

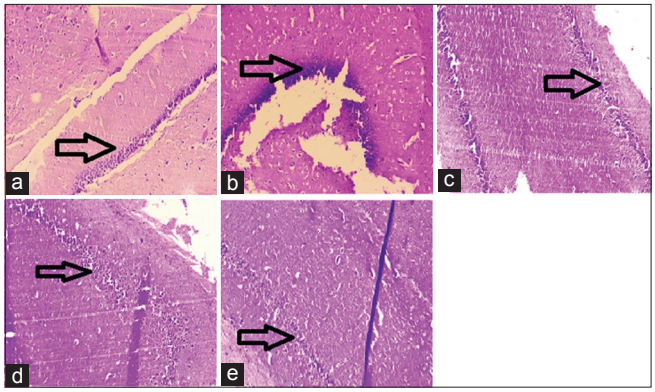

Figure 3: Figure shows ×10, H and E stained histology of hippocampus, pyramidal layer neuronal cell bodies of CA-1 region, (a) (normal control) shows clear neuronal morphology, (b) (stress group) shows a decrease in hippocampal pyramidal layer thickness, (c) vitamin C-treated group had increased number of surviving neurons after receiving chronic stress, (d and e) received beta-carotene and caffeine, respectively, and both the groups showed decrease in apical dendrites of pyramidal neurons

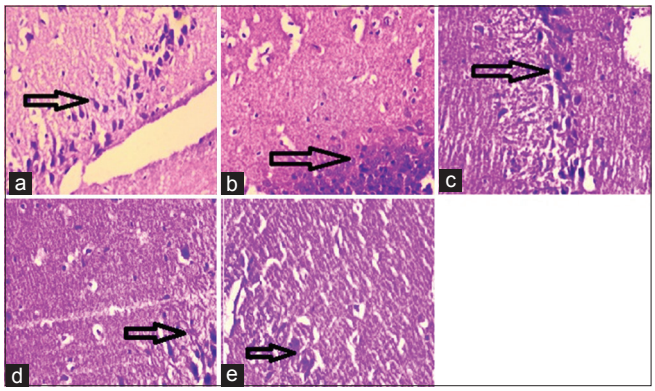

Figure 4: Figure shows ×40, H and E stained histology of hippocampus, pyramidal layer neuronal cell bodies of CA-1 region, (a) (normal control) shows clear neuronal morphology and arrow pointing the apical dendrites of pyramidal neurons extend into stratum radiatum layer of hippocampus, (b) (stress group) shows a decrease in hippocampal pyramidal layer thickness, (c) vitamin C-treated groups had increased number of surviving neurons and well-developed apical dendrites after receiving chronic stress, (d and e) (beta-carotene and caffeine groups, respectively) showed decrease in apical dendrites of pyramidal neurons

of calcium causes postsynaptic NGF release[12] which then leads to an increase in presynaptic vesicle cycling.[13] These mechanisms all serve to support LTP and synaptic plasticity. SVCT 2 is found frequently in dense regions of the neurons in the brain such as the hippocampus and cortex where the issue is related to the regional distribution of ascorbate in the brain.[14]

Marcos Roberto de Oliveira et al. showed vitamin A supplementation for 28 days induced cognitive decline, decreased BDNF levels, impaired mitochondrial function, nitrosative stress, and endoplasmic stress in animals.

By decreasing BDNF levels, vitamin A impairs not only neuronal plasticity that is necessary for learning and memory processes but also the ability of this organelle to produce adequate amounts of Adenosine triphosphate (ATP) needed to counteract acute and chronic stress. In fact, it was demonstrated that vitamin A affects mitochondrial function *in vitro* and *in vivo*.[15] However, Kheirvari et al. has concluded that high dose of vitamin A may increase BDNF and NGF levels and low dose may decrease BDNF and NGF.[16] The effect would be more pronounced when it is administered along with vitamin E or vitamin C. Studies have reported variable effect of caffeine on LTP and memory whereas the majority studies report LTP and memory enhancement with caffeine[17,18] and few studies report no effect,[19,20] or few even show memory impairment.[21] However, the present study did not show clear morphology of hippocampal pyramidal neurons probably due to higher caffeine doses might be associated with a decreased density of cortical A1R and hippocampal BDNF levels.[22] However, an extensive study will be carried out further to find the effect of caffeine with different dosage of caffeine.

CONCLUSION

This study suggests that oral administration of vitamin C prevents oxidative stress and enhances synaptic activity and cognitive function, therefore, induce neuroprotection. Vitamin C found to be superior when compared other antioxidants and probably be used as natural dietary resources to improve memory and combat stress.

REFERENCES

1. Sleimana SF, Chao MV. Downstream consequences of exercise through the action of BDNF. Brain Plast. 2015;1:143-8.
2. Whittemore SR, Seiger A. The expression, localization and functional significance of beta-nerve growth factor in the central nervous system. Brain Res. 1987;434(4):439-64.
3. Cimino M, Cattabeni F, Di Luca M, Peruzzi G, Andena M, Tirassa P, et al. Levels of NGF, p75NGFR and ChAT immunoreactivity in brain of adult and aged microencephalic rats. Neurobiol Aging. 1996;17(1):137-42.
4. McGaugh JL. Time-dependent processes in memory storage. Science. 1966;153(3742):1351-8.
5. Conrad CD. Chronic stress-induced hippocampal vulnerability: The glucocorticoid vulnerability hypothesis. Rev Neurosci. 2008;19(6):395-411.
6. Sapolsky RM. Stress, the Aging Brain, and the Mechanisms of Neuron Death. Cambridge, MA: MIT Press; 1992.
7. Gama CS, Kunz M, Magalhães PV, Kapczinski F. Staging and neuroprogression in bipolar disorder: A systematic review of the literature. Rev Bras Psiquiatr. 2013;35:70-4.
8. Nawar WF. Lipids. In: Fennema O, editor. Food Chemistry. 3rd ed. New York: Marcel Dekker, Inc.; 1996. p. 225-320.
9. Iwata E, Asanuma M, Nishibayashi S, Kondo Y, Ogawa N. Different effects of oxidative stress on activation of transcription factors in primary cultured rat neuronal and glial cells. Brain Res Mol Brain Res. 1997;50(1-2):213-20.
10. Zou J, Crews F. CREB and NF-kappaB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Cell Mol Neurobiol. 2006;26(4-6):385-405.
11. Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci. 2004;19(7):1699-707.
12. Hartmann M, Heumann R, Lessmann V. Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. EMBO J. 2001;20(21):5887-97.
13. Walz , Jungling K, Lessmann V, Gottmann K. Presynaptic plasticity in an immature neocortical network requires NMDA receptor activation and BDNF release. J Neurophysiol. 2006;96(6):3512-6.
14. Wilson JX. Vitamin C transport in animals and plants. In: Asard H, May J, Smirnoff N, editors. Vitamin C-Functions and Biochemistry in Animals and Plants. Oxford: BIOS Scientific Publisher Ltd.; 2003. p. 97-117.
15. Oliveira MR. The neurotoxic effects of vitamin A and retinoids. An Acad Bras Cienc. 2015;87 2 Suppl:1361-73.
16. Kheirvari S, Uezu K, Yamamoto S, Nakaya Y. High-dose dietary supplementation of vitamin A induces brain-derived neurotrophic factor and nerve growth factor production in mice with simultaneous deficiency of vitamin A and zinc. Nutr Neurosci. 2008;11(5):228-34.
17. Lu KT, Wu SP, Gean PW. Promotion of forskolin-induced long-term potentiation of synaptic transmission by caffeine in area CA1 of the rat hippocampus. Chin J Physiol. 1999;42(4):249-53.
18. Martín ED, Buño W. Caffeine-mediated presynaptic long-term potentiation in hippocampal CA1 pyramidal neurons. J Neurophysiol. 2003;89(6):3029-38.
19. Furusawa K. Drug effects on cognitive function in mice determined by the non-matching to sample task using a 4-arm maze. Jpn J Pharmacol. 1991;56(4):483-93.
20. Hudzik TJ, Wenger GR. Effects of drugs of abuse and cholinergic agents on delayed matching-to-sample responding in the squirrel monkey. J Pharmacol Exp Ther. 1993;265(1):120-7.
21. Sansone M, Battaglia M, Castellano C. Effect of caffeine and nicotine on avoidance learning in mice: Lack of interaction. J Pharm Pharmacol. 1994;46(9):765-7.
22. Ardais AP, Borges MF, Rocha AS, Sallaberry C, Cunha RA, Porciúncula LO. Caffeine triggers behavioral and neurochemical alterations in adolescent rats. Neuroscience. 2014;270:27-39.

How to cite this article: Sangeetha A, Mohan SK, Kumaresan M. Chronic administration of vitamin C increases cognitive function in chronic stress-induced rats. Natl J Physiol Pharm Pharmacol 2017;7(11):1190-1194.

Source of Support: Nil, **Conflict of Interest:** None declared.