Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2010, 1 (2) : 71-76
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233

Anti-oxidant activity of Vitamin B complex on stress-induced neurobehavioral changes in rats

Nachiket R. Amin*, Shailesh K. Pallewar and Guno S. Chakraborthy

Department of Pharmacognosy, School of Pharmacy & Technology Management, Narsee Munjee Institute of Management Studies, Shirpur, Maharashtra, India

---

Abstract

Research in effect of restraint stress (RS) currently includes various cellular, molecular, genetic, clinical, therapeutic approaches. Restraint stress and its modulation were studied for elevated plus maze (EPM) and its biochemical parameters with respect to antioxidants. Restraint stress (RS) for 1 hr reduced the number of open arm entries indicating enhanced anxiogenic response in the EPM test as compared to normal non RS group of rats. Pretreatment with vitamin B complex (100 and 200 mg/kg) group attenuated this RS induced effects. Furthermore, lipid peroxidation (enhanced MDA levels), a hallmark of oxidative tissue injury, has been found to be elevated in neurodegenerative disorders such as Alzheimer's disease (AD). Hence it is thought that oxidative stress may be an underlying mechanism in AD, and agents that prevent oxidative damage may be particularly efficacious in the treatment of AD. MDA is sensitive marker for lipid peroxidation which suggests that RS induced neurobehavioral changes are probably associated with increased free radical generation. Biochemical data showed that RS enhanced MDA levels in serum and this were attenuated after pretreatment with the help of antioxidant. The pharmacological and biochemical results indicate that free radicals might be involved in such stress- induced neurobehavioral effects.

Keywords: Oxidative stress, Elevated plus maze, Antioxidants, Free radicals.

INTRODUCTION

The Central Nervous System is a crucial mediator during certain stress related responses and some limbic structures, particularly amygdaloid complex and its interaction with lower brain stem areas, which have been implicated [1]. The concept of stress has evolved into one of a

"stress systems" wherein complex interactions between central nervous system viz. limbic system, hypothalamus-pituitary-adrenal (HPA) axis and several components of visceral system occur in response to a variety of stressful inputs [5]. Stressful stimuli can disrupt the physiological homeostasis and inabilities to cope with such aversive inputs have widespread deleterious effects on the biological system. Emotional and environmental stressors reportedly influence brain function and is known to be as key factor in the genesis of neuropsychiatric disorders [2, 3]. Exposure to such stressors are known to evoke responses such as reduced locomotor activity marked anorexia, decreased growth rate and hypertension [4]. Such interactions play a significant role in the outcome of the stress response and are crucial determinants of health and disease [5-9]. Complex neurochemical mechanisms are also known to regulate the activity of stress system and drugs modulating a variety of neurotransmitters/neuromodulators exert differential effects on the response of various organs and organ systems [10-13]. Furthermore, lipid peroxidation, a hallmark of oxidative tissue injury, has been found to be elevated in neurodegenerative disorders such as Alzheimer's disease (AD) [14]. Hence it is thought that oxidative stress may be an underlying mechanism in AD, and agents that prevent oxidative damage may be particularly efficacious in the treatment of AD [15].

Reactive oxygen metabolites/species are produced during normal cellular metabolic functions and contains super oxide anion (O^{2-}), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) [16, 17]. The crucial factor in neurodegenerative processes, including cell death, motor neuron disease, and axonal injury is antioxidant/ pro-oxidant balance. Also there is direct or indirect protection which is provided by antioxidants to cells from adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions [18, 19]. Elevated plus maze (EPM) test is a sensitive behavioral test to evaluate anxiety/ stress and restraint stress is a widely used model to induce emotional stress in rodents [1, 11-13]. The above present study was designed to evaluate the possible involvement of free radicals in stress induced neurobehavioral changes using EPM. Apart from it malondialdehyde (MDA) levels were also determined which lead to the lipid peroxidation estimation in serum for corroborative purposes.

MATERIALS AND METHODS

Drugs and chemicals- The drugs used were: Vitamin B complex with B_{12} (Merck Ltd, India). Vitamin B complex was dissolved in water. The experimental group received antioxidants for five consecutive days following which they were exposed to RS. The control group received vehicle i.e. distilled water in it. All drugs were freshly prepared and administered orally at a concentration of 10mg/ml. Immediately after RS; the rats were tested for neurobehavioral effects.

Experimental animals- Male Wistar rats (150-200g) were used for the study. The animals (n=5per group) were maintained under standard laboratory conditions (12h light/dark cycle at $22^0 \pm 2^0$ C) and had free access to food and water throughout the experiments. Animal care was taken as per Indian National Science Academy (INSA) Guidelines for Care and Use of Animals in Scientific Research. The study protocol was approved by Institutional Animal Ethics Committee (IAEC).

Stress procedure- Restraint stress (RS) for 1 hr at room temperature was used as the experimental stressor. For this purpose animals were immobilized in adjustable Plexiglas restrainers (INCO, Ambala), which were well-ventilated devices. Immediately after the restraint stress procedure, rats were exposed to behavioral tests for 5 min. After the behavioral tests, the blood was collected from retro-orbital plexus and serum was separated for MDA estimation.

Neurobehavioral studies

Elevated plus maze test- The elevated plus maze consisted of two open arms 40×40 cm, crossed with two similar closed arms with wall of 40cm height. The arms were connected so that the maze had a look of plus sign. The entire maze was elevated 50cm above the ground level and placed in a quiet and dimly lit room[20, 21]. Vehicle or drug treated rats were placed individually in the center of the maze facing the closed arms. The following parameters were measured: numbers of open arm entries and time spent in the open arms and similar observations were also made for the closed arms. The percentage of open arm entries was calculated from open arm entries divided by the total number of entries in both open and closed arms. The time spent on open-arm exploration divided by total time spent in both open and closed arms were also calculated.

Biochemical studies

MDA estimation in blood- The serum samples were analyzed for the lipid peroxidation by the method of Okhawa *et al*[22]. In this method, 0.5 ml of the serum samples and 2.5 ml TCA (20%) were taken in the test tubes. After centrifugation at 3500 rpm for 10 min, the supernatant was decanted and precipitate was washed once with 2.0 ml of (0.05*M* H_2SO_4). Washed precipitate was dissolved in 2.0 ml of H_2SO_4 and 3.0 ml of thiobarbituric acid (0.68% TBA in Na_2SO_4) was added and vortexed. The tubes were incubated in boiling water bath for 30 min. The tubes were taken out and kept under running tap water. To this chromogen 4.0 ml of *n*-butanol was added with vigorous shaking. Centrifugation was done at 3000 rpm for 10 min. The upper organic layer was separated and absorbance was taken at 530 nm using UV- visible spectrophotometer (Lambda-25, Perkin Elmer). The calculations were carried out using extinction coefficient and results were expressed in n mol/ml.

Statistical analysis- The data were expressed as mean ± SEM and analyzed using a one way analysis of variance (ANOVA) followed by Dunnett's tests for inter group comparison. Biochemical data was analyzed by Mann- Whitney 'U' test. *P* value at least 0.05 was considered as the level of significance in all statistical tests.

RESULTS

Effect of restraint stress (RS) and antioxidants in elevated plus maze (EPM) test- Analysis of EPM test data revealed that the per cent (%) number of entries in open arms were significantly different across all groups ($P<0.01$- one way ANOVA). As shown in Table 1, RS induced a significant reduction in the per cent open-arm entries in EPM, as compared to controls (no RS) ($P<0.05$, Dunnett's test).

Table 1- Effect of restraint stress (RS) and antioxidants on Elevated plus maze (EPM) test parameters in rats [Values are mean ± SEM]

Treatment (mg/kg i.p)	EPM parameters	
	Open arm entries (%)	Open arm time (%)
Vehicle (no RS)	25.8 ± 3.3	15.2 ± 3.7
Vehicle + RS	8.2 ± 4.2	5.2 ± 2.6
Vit B comp (100)	29.3 ± 2.1*	17.3 ± 3.4
Vit B comp (200)	31.1 ± 2.3*	19.1 ± 2.9

Vit B comp (100)**:** Vitamin B complex (100mg/kg); Vit B comp (200)**:** Vitamin B complex (200mg/kg); *P<0.05 (compared to vehicle + RS group)

Restraint stress effect on MDA levels in serum

Biochemical analysis of serum levels showed that MDA level is more in RS exposed animals as that of non RS group. Initial treatment with vitamin B complex notably reduced increased MDA level in serum compare to RS exposed group.

Table 2- Effect of restraint stress (RS) and antioxidants on malondialdehyde (MDA) levels in serum of rats [Values are mean ± SE]

Treatment (mg/kg i.p)	MDA levels in Serum (n moles/ ml)
Control (no RS)	2.8 ± 0.3
RS	5.0 ± 0.2
Vitamin B complex (100) + RS	3.8 ± 0.3
Vitamin B complex (200) + RS	3.3 ± 0.2

DISCUSSION

Emotions and stress, behavioral factors, are responsible for changes in neurobehavioral profile of an individual and responsible for induction of anxiety. In EPM number of entries and/or time spent in open arm are indicators of anxiolytic activity. At dose level of 100 and 200mg/kg vitamin B complex significantly reversed such RS- induced changes in open arm entries in EPM. Reversal of the RS-induced EPM test parameters with the pretreatment with vitamin B complex suggested that free radical generation in the CNS during RS might be responsible for such stress-induced suppressed (anxiogenic) behavior. Restraint stress is mainly used for induction of stress and present findings showed that vitamin B Complex dose reversed RS induced reduction in open arm entries in EPM test. This suggests that free radicals that are generated in CNS during RS might be responsible for such anxiogenic behavior. MDA is sensitive marker for lipid peroxidation which suggests that RS induced neurobehavioral changes are probably associated with increased free radical generation. Prior treatment with vitamin B complex significantly reduced increased MDA level in serum compare.

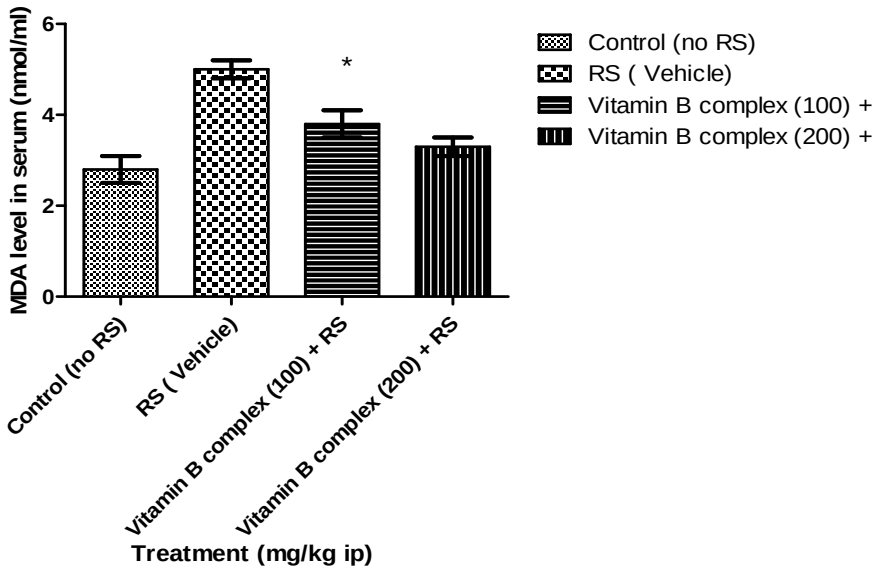

Fig. 1. *In vivo* changes in lipid peroxidation (MDA levels) in different groups of rat serum
A significant decrease in the RS and Vitamin B complex (100mg/kg) treated group ($p < 0.05$) was found when compared to the RS (Vehicle) treatment alone.*

CONCLUSION

Experimental pharmacological studies have shown that anti- anxiety agents attenuate a variety of autonomic visceral and immunological responses to stress. Elevated plus maze apparatus can be used effectively to measure neurobehavioral profile in animals under influence of anxiolytic agents. Role of Free radicals and antioxidants defense mechanism are proposed to play important role in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Biochemical data showed that RS enhanced MDA levels in serum and this were attenuated after pretreatment with the help of antioxidant. After taking biochemical and pharmacological data together indicate that emotional stress may induce generation of free radicals in CNS and this is responsible for anxiogenic behavior that is lowered by vitamin B Complex combinations. Biochemical and pharmacological data clearly showed that emotional stressors may induce free radical generation in the CNS, which might result in anxiogenic behavior, which are attenuated by antioxidants.

Acknowledgement
The authors are grateful to the Department of Pharmacology for their support in this project.

REFERENCES

[1] Henke P G, Ray A & Sullivan R M, *Dig Dis Sci* 36 (**1991**) 1633.
[2] Selye H, *Nature*, 13 (**1936**) 32.
[3] Chrouses G P & Gold P W, *JAMA*, 267, (9) (**1992**) 1244.
[4] Bohus B, Koolhas J M, Korte M, Bouws G A H, Eisenga W & Smit J, *Neurosci Biobehav* 14 (**1990**) 529.

[5] Burchfield SK, Wood SC & Elich MS, *physiol Behav*, 24 (**1988**) 297.
[6] Cancela LM, Rossi S & Molina VA, *Brain Res Bull*, 26 (**1991**) 671.
[7] Irwin J, Ahluwalia P & Anisman H, *Psychopharmacology*, 90(**1996**)203.
[8] Stone E A & Platt J E, *Brain Res*, 237 (**1982**) 405.
[9] Stone E A, Slucky A V, Platt J E & Trullas R, *J Pharmacol Exp Ther*, 233(**1985**) 382.
[10] Carli M, Prontera C & Samanin R, *Neuropharmacology*, 28 (**1989**) 471.
[11] Henke P G & Ray A, *Acta physiol Hung*, 80 (**1992**) 117.
[12] Ray A, Henke P G & Sullivan R M, *Physiol Behav*, 24 (**1988**) 259.
[13] Ray A, Mediratta P K & Sen P, *Physiol Behav*, 51 (**1992**) 293.
[14] Subbarao KV, Richardson JS, Ang LC: *J Neurochem*, **1990**, 55, 342-345.
[15] Smith MA, Hirai K, Nunomura A, Perry G: *Drug Dev Res*, **1999**, 46, 26–33.
[16] Halliwell B, *Nutr Rev*, 5 (**1994**) 253.
[17] Halliwell B & Gutteridge J M C, Free radicals in biology and medicine. (Oxford university press, New York), **1999**.
[18] Halliwel B, *The Lancet*, 344 (**1994**) 721.
[19] Wulf D, *Physiol Rev*, 82 (**2002**) 47.
[20] Pellow S, Chopin S E, File S E & Briley M, *J Neurosci Methods*, 14 (**1985**) 140.
[21] Bhattacharya S K & Satyan K S, *Indian J Exp Biol*, 35 (**1997**) 565.
[22] Masood A, Banerjee B, Vijayan V K & Ray A, *Eur J Pharmacol*, 458 (**2003**) 135.