Indian J.L.Sci.2(1) : 27-30, 2012

A STUDY ON THE ROLE OF ANTIOXIDANT VITAMIN E SUPPLEMENTATION ON BEHAVIORAL CHANGES INDUCED BY IMMOBILIZATION STRESS IN MICE

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ABSTRACT

Stress is a term used to describe a state characterized by a broad range of physiological and behavioral changes resulting from one or more stressors. The present study was undertaken to investigate the potential of the antioxidant vitamin E known as a powerful free radical scavenger. The study group consists of Group I) control Group II) control stress group III) mice supplemented with vitamin E group IV) mice supplemented with vitamin E subjected to immobilization stress. Animals subjected to immobilization by placing them in wire mesh cage for their size about 6 hours. The behavioral assessment in open field showed a significant increase in immobilization time with a decrease in rearing, grooming and ambulation behaviour. In elevated plus maze there is a significant increase in transfer latency with decrease in time on open arm and numbers of arms crossed were observed. In our study animals treated with vitamin E showed a better tolerance to immobilization stress by improvement in behavioral changes compared to stress group.

KEYWORDS : Immobilization stress, vitamin E, open field, elevated plus maze

Today's man is exposed to large number of stress from a variety of sources. The imbalance between pro-oxidant and antioxidant gives rise to cellular oxidative stress (Oski , 1980). This stress cause lipid peroxidation that leads to many pathologies. This lipid peroxidation also alters behaviour of animal and human beings. Biologically stress was defined as "the non-specific response of the body to any demand". Selye also found the triphasic response to a wide variety of stressors. The initial response he termed as the "Alarm reaction" the enhanced state of recovery as the "State of Resistance" and the "state of Exhaustion" when resistance fails. He labeled this triad as General Adaptation Syndrome (G-A-S). A stressful situation induces the organism to mobilize not only the adrenal system but also the central nervous system and the pituitary. Restraint stress constitutes a high stressor than either handling or isolation of animals as it induces the release of a greater quantity of corticosteroids (Djordjevi'c et.al, 2003). Restraint stress affect the para ventricular nucleus of hypothalamus known to be a key site in the activation of the Hypothalamo Pituitary Adrenal (HPA) axis. To attenuate the effects of stress the role of antioxidants are proved to be effective. Antioxidants such as vitamin E is a powerful free radical scavenger.Vitamin E is naturally occurring lipid soluble, chain breaking antioxidant protecting cell membranes from per oxidative damage (Nahed and Sahar, 2007). As a result of literature investigations carried out, there are ample of works has been done on vitamin E against stress but a very few number is discussed regarding behavioral changes during stress.

MATERIALS AND METHODS

The study was carried out under four groups with six animals each (n = 6) using male swiss albino mice weighing about 20 -25gms. All experiments performed were approved by the Institutional Animal Ethics committee and care of experimental animals was taken as per CPCSEA guidelines.

Group I : Control animals to study the baseline data

Group II: Control stress group

Group III: Animals treated with vitamin E at a dose of 100mg / kg body weight orally for four weeks.

Group IV: Animals subjected to Immobilization stress after treated with vitamin E for 4 weeks.

Stress Procedure

The animals were subjected to Immobilization stress to assess the effect of antioxidants.

Immobilization Stress

The animals were immobilized by placing them in a wire mesh cages of their size for a period of 6 hours (Dhir et al.,2006 ; Francolin and Almeida ,2004).

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Behavioral Parameters

The changes in behaviour of mice following acute stress were evaluated by open field method and elevated plus maze method.

Open Field

The field was a closed area which is divided into 25squares equally. The 100 W frosted bulb was placed above the field during the activity testing. The behavioral parameters of each mice were tested in a wake condition in open field for 3 minutes by placing the animals at the corner of the apparatus. The parameters are

I) Immobilization Time

It is the duration of time that mice were, holding its head against the gravity but without movements of head, body or limb with opened eyes.

II) Grooming

Rhythmic paw movement over the face and or head for face rubbing include episodes of biting and cleaning of paws.

III) Rearing

Standing still on upright on its hind limb only

IV) Ambulation

When all the four limbs were in one particular square (central or peripheral) of the open field (Bhattacharya and Satyan,1997 and Takayoshi et.al, 2006).

Elevated Plus Maze

The maze had two open arms (50 cm X 10 cm) and at right angle to it, two crossed arms (50 cm X 10 cm X 40 cm) with the roof uncovered an open central crossing (10 cm X 10 cm) and was rising to a height of 50 cm. The behavioral parameters of each mice were tested for 5 minutes in wake condition in Elevated Plus Maze by placing them at the end of an open arm are i)Transfer latency: Time taken by the animal to move from the outer end of the open arm to either of two closed arm ii)% time in open arm: The percentage of total testing time spent in the open arm iii)% time in closed arms: The percentage of total testing time spent in the closed arm iv)Number of crossing of the arms: The number of times the animal crosses the centre for going one arm to any other of three arms.

RESULTS

The data of behavioral analysis following acute immobilization stress with respect to control group analyzed is given in the table below.

Exposure to immobilization stress show significant changes in animal behaviour in open field and elevated plus maze. In open field (Table, 1) data shows significant increase in immobilization ($P< 0.05$) accompanied with decrease in rearing ($P< 0.05$) grooming ($P< 0.05$) and ambulation behaviour such as peripheral squares ($P< 0.05$) and central squares. In elevated plus maze (Table, 2) there is significant increase in transfer latency ($P< 0.05$) with time spent in closed arms ($P< 0.05$) and decrease in time spent on open arms ($P< 0.05$) and number of arms crossed ($P< 0.05$). This immobilization stress induced change in the peripheral, central, immobilization and rearing were significantly ($P< 0.05$) prevented in vitamin E treated immobilization stress exposed group.

DISCUSSION

In this present study, Acute Immobilization stress on swiss albino mice, constitute a high stressor as it induced the release of a greater quantity of corticosteroids, it is understood that stress cause the production of free radicals which affects all the cells, organisms and leads to lipid peroxidatioan. This can be controlled and its further damage can be reduced by antioxidants in the plasma and tissues . In

Table 1: Behavioral Parameters in Open Field in control, control stress and vitamin E administered Mice

S.No	Parameters in Open field	Control group	Control Stress	Vit.E. Treated with immobilization stress	'P'-Value
1	Immobilization time (sec)	35.83 ± 1.50	137.66 ± 1.60	82.33 ± 2.50	P < 0.001
2	Grooming	69.66 ± 0.38	33.66 ± 1.24	60.16 ± 1.27	P < 0.05
3	Rearing	6.00 ± 0.11	2.83 ±0.14	4.16 ±0.11	P < 0.05
4	Ambulation Central	3.01 ± 2.60	1.50 ± 1.32	2.66 ± 0.74	P < 0.05
5	Ambulation Peripheral	46.83 ± 0.23	17.16 ± 0.26	35.33 ± 0.34	P < 0.05

Table 2: Behavioral Parameters in Elevated plus maze in control, control stress and vitamin E administered Mice

S.No	Parameters in Elevated plus maze	Control group	Control Stress	Vitamin E Treated with immobilization stress	'P'-Value
1	Transfer latency	8.66 ± 2.80	62.04 ± 4.30	31.45 ± 2.80	P < 0.05
2	Time spent in open arm (sec)	156.13 ± 0.54	52.22 ± 0.76	102.00 ± 0.80	P < 0.05
3	Time spent in closed arm (sec)	39.50 ± 21.10	203.00 ±2.80	122.66 ± 3.60	P < 0.001
4	Number of crossings	18.00 ± 0.13	0.08 ± 0.14	15.66 ± 0.10	P < 0.01

Values are expressed as mean + SD
Values are taken as a mean of six individual experiments
P* < 0.05 P** < 0.01 P*** < 0.001

our study stress caused behavioral alteration in open field and elevated plus maze study. This may be due to the action of paraventricular nucleus of hypothalamus which activates the Hypothalmo Pituitary Adrenal Axis for the stimulating the ACTH from Anterior Pituitary. This ACTH rise increases the lipid peroxidation and also increases the non-specific permeability against Ca^{+2} ions and so the degradation of membrane function. Corticosterone not only stimulates the Pituitary but also increases sympathetic outflow to the adrenals resulting in increased output of both cortical and medullary hormones that may cause behavioral abnormalities in albino mice (Sinha and Ray ,2004). The behavioral changes produced by acute immobilization stress correlated will with the earlier reports of Menon and Dandiya (Menon and Dandiya, 1969) showing that the increase in immobilization time in Open-Field and Transfer Latency in elevated plus maze was significant. Along with these changes, decrease in rearing, grooming and ambulation also revealed that acute immobilization caused fatigue and altered the excitability of the nervous system. These alteration in nervous system might be due to decreased prostoglandins levels which influences the Serotonin secretions. This has been said to maintain physiological and Psychic homeostasis during stress (Bhattacharya and Bhattacharya,1982) In our study Vitamin E treated animals showed better tolerance to immobilization stress and this correlates well with previous study by Yargicoglu.P (Yargicoglu and Piraye ,2003 ; Ebrahim et al.,1995), were animals pretreated with vitamin E was found to be effective in over coming stress significantly by decreasing the corticosterone level .

CONCLUSIONS

This study quantifies the relationship between the acute immobilization stress and behavioral phenomenon with respect to the antioxidant vitamin E. Our results clearly proves the role of vitamin E, a powerful lipid-soluble antioxidant which improves the behavioral changes of the animals after immobilization stress.

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