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International Journal of Health Research, September 2008; 1(3): 151-162

Available at http://www.ijhr.org

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Original Research Article

Possible nitric oxide modulation in the protective effect of trazodone against sleep deprivation-induced anxiety like behavior and oxidative damage in mice

Received: 17-Jul-08 Revision received: 01-Sep-08 Accepted for publication: 19-Sep-08

Abstract

Purpose: *The present study was designed to explore the possible nitric oxide modulation in the protective effect of trazodone against sleep deprivation-induced behavioral alterations and oxidative damage in mice.*

Methods*: In a controlled study, sleep deprivation was induced in 10 groups of mice (6 in each group) for 72 hr by using grid suspended over water method. Trazodone (5 and 10 mg/kg, ip), L-arginine (50 mg/kg, ip), L-NAME (10 mg/kg, ip) and methylene blue (10 mg/kg, i.p) were administered for 5 days, 2 days prior to the 72 hr sleep deprivation. Various behavioral tests (plus maze, zero maze, mirror chamber, actophotometer) followed by oxidative stress parameters (malondialdehyde level, reduced glutathione, catalase, nitrite and protein) were assessed in the animals.*

Results: *The trazodone treatment significantly indcued anti-anxiety like effect, improved locomotor activity and antioxidant effect as indicated by reduced lipid peroxidation, nitrite concentration and restoration of depleted reduced glutathione and catalase activity. Further, prior treatment of the animals with L-NAME and methylene blue potentiated the protective effect of trazodone (5 mg/kg) ($p<0.05$). However, L-arginine combined with trazodone (5mg/kg) reversed the protective effect of trazodone ($p<0.05$).*

Conclusion*: Results of present study suggest that NO modulation is involved in the protective effect of trazodone against sleep deprivation-induced anxiety like behavior and oxidative damage in mice.*

Keywords: *Anxiety, locomotor activity, oxidative stress, sleep deprivation, trazodone, L-arginine, L-NAME, methylene blue.*

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Introduction

Depression is commonly associated with poor quality of sleep and forms an essential criterion for the diagnosis of the condition. Studies have also suggested that up to 90% of patients suffering from major depressive disorders experience some type of sleep disruption[1,2]. Persistent sleep disturbances are also a risk factor to depression[3]. Disturbance of sleep is a common problem in depressed patients and antidepressant drugs frequently been prescribed to manage sleep related problem in such patient[4]. Sleep deprivation aggravates health risk factors including depression, anxiety[5,6], cognitive dysfunctions[7,8,9], impaired motor activity[10], behavioral irritability and poor performance. Sleep deprivation weaken anti-oxidative[11,13] defense capacity. It also impairs antioxidant defense, leading to oxidative damage by causing imbalance between oxidants and antioxidants[13].

Lower doses of antidepressants are frequently prescribed to manage insomniac patients without depression[14]. Tricyclic antidepressants (TCAs) have been reported to improve sleep continuity in such patients[15] whereas most selective serotonin reuptake inhibitors (SSRIs) may exhibit alerting effects, thereby reducing TST and sleep efficiency, and increasing wakefulness[15,16]. Trazodone is a second-generation antidepressant which blocks 5-HT_2 receptors. However, its scientific and mechanistic basis of antidepressants use in the treatment and management of sleep deprivation is not understood properly so far. Further, no adequate information available to understand the mechanism of their clinical potential in sleep deprivation. However, complex interactions of oxidative stress and nitric oxide have been suggested in sleep regulation. Within the later dorsal tegmentum (LDT), pedunculopontine tegmentum and dorsal rephe nucleus, nitric oside (NO)-containing neurons overlap and grouped according to their contribution to sleep mechanisms[17]. The main target for NO is the soluble gunaylate cyclase that triggers overproduction of cyclic guanosine monophoshate. In neurons of the pontine tegmentum, NO facilitates sleep and has been implicated in several oxidative neurodegenerative disorders[18,19].

Despite extensive research on the beneficial effects of trazodone and NO modulators in various disease conditions, limited studies explored the possible therapeutic potential of antidepressant in the treatment of sleep deprived-induced behavioral alterations and oxidative stress. Therefore, the present study has been designed to investigate the possible nitric oxide modulation in the protective effects of trazodone against sleep-deprivation-induced anxiety like behavior and oxidative stress.

Materials and Methods

Animals

Albino mice weighing 22 – 30 g, bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used for this study. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. They were acclimatized to laboratory conditions before the experiment. The animals were divided into 10 groups consists of 6 animals in each group. All experiments were carried out in daylight. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs and treatment schedule

Animals were divided into different groups. Naïve (saline) and control (sleep deprived) were treated as groups 1 and 2. Animals that received trazodone (5 mg/kg, 10 mg/kg), L-arginine (50 mg/kg, i.p), L-NAME (10 mg/kg, i.p) and methylene blue (10 mg/kg, i.p) were

treated as groups 3 to 7, respectively, while those that received L-arginine (50 mg/kg, i.p), L-NAME (10 mg/kg, i.p) and methylene blue (10 mg/kg, i.p) after 10 min pretreatment with trazodone (5 mg/kg) were treated as group 8 to 10, respectively. Trazodone, L-arginine, L-NAME and methylene blue were freshly prepared in distilled water and administered intraperitoneally for 5 days, 2 days prior to 72 hr sleep deprivation.

Sleep deprivation

The animals were sleep deprived for 72 hr by placing them on grid suspended over water, based on modified method of Sinomiya et al[20]. Briefly, they were placed on a grid floor (29×15×7 cm) inside the plastic cage filled with water to 1 cm below the grid surface for 72 hr. The stainless steel rods of the grid (3 mm wide) were set 2 cm apart from each other. Food and water were provided *ad libitum.*

Behavioral assessments

Anxiety levels were measured using the following approaches*:*

Elevated plus maze test— Developed by Pellow and File[21], elevated plus maze is a novel test for testing selective anxiogenic and anxiolytic drug effect in rodents. The plus maze apparatus consisted of two open (16 x 5 cm) and two closed arm (16 x 5 x12 cm) and placed at a height of 25 cm for mice. The animal were placed individually at the centre of the elevated plus maze with their head facing toward an open arm. During the 5 min test, average time spent per entry in open arm of the maze was recorded.

Zero maze test—The Zero maze described by Shepard[22] is a modification of elevated plus maze model of anxiety in rodents. The maze comprised of black perspex annular platform (105 cm diameter, 10 cm width) elevated to 65 cm above ground level, divided equally into four quadrants. Black Perspex walls (27 cm height) on both the inner and outer edges of the platform enclosed two opposite quadrants; the remaining two quadrants were surrounded by Perspex lip (1 cm in height). An animal was placed in the closed quadrant. During the 5 min test, the average time spent per entry in open quadrant of the maze was recorded.

Mirror Chamber Test—Animals were placed individually at the distal corner of a mirror chamber at the beginning of the test. The mirror chamber consisted of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session, the latency to enter the mirror chamber and average time spent per number of entries in mirror chamber were recorded. An anxiogenic response was defined as the reduced number of entries and time spent in the mirror chamber[23].

Measurement of locomotor activity— Ambulatory movements were recorded by using actophotometer (IMCORP, India). The apparatus was placed in a darkened, light - sound attenuated and ventilated testing room. Before locomotor task, animals were placed individually in the activity meter for 3 min. The ambulatory movements were recorded using actophotometer for a period of 5 min and expressed in terms of total photo beam counts for 5 min per animal[24].

Biochemical tests

Tissue preparation

Animals were sacrificed by decapitation on the same day following behavioral assessments. The brains were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by

centrifugation of the homogenate at 12000 ×g for 20 min at 4˚ C.

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in the whole brain was measured according to the method of Wills[25]. The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. Results were expressed as moles of malondialdehyde per milligram protein.

Estimation of reduced glutathione

Reduced glutathione in the brain was estimated according to the method of Ellman[26]. A 1.0 ml of homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid by keeping the mixture at 4 ℃ for 1 hr and the samples were immediately centrifuged at 1200 ×g for 15 min at 4 ℃. The assay mixture contains 0.1 ml of supernatant, 2.7 ml of phosphate buffer of pH 8.0 and 0.2 ml of 0.01 M dithiobisnitrobenzoic acid (DTNB). The yellow color developed was read immediately at 412 nm using Perkin Elmer Lambda 20 spectrophotometer. Results were expressed as micromole GSH per milligram protein.

Nitrite estimation

Accumulation of nitrite was measured in cell free supernatants from brain homogenates by spectrophotometer assay based on Greiss reagent 15 (1% sulphanilamide 0.1% naphthylethylenediamine dihydrochloride 2.5% phosphoric acid) incubated at room temperature for 10 min to yield a chromophore. Nitrite is the stable product of nitric oxide (NO) in living system. Absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micro molar nitrite per milliliter homogenate[27].

Protein estimation

The protein content was measured according to the method of Lowry[28] using bovine serum albumin as standard.

Catalase estimation

Catalase activity was assayed by the method of Luck 1971[29], wherein the breakdown of hydrogen peroxides (H_2O_2) was measured at 240 nm. Briefly, assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%). The change in absorbance was recorded at 240 nm and the results were expressed as micromole H_2O_2 decomposed per milligram of protein/min.

Statistical analysis

All the values are expressed as mean ± SEM. The data were analyzed using analysis of variance (ANOVA) followed by Tukey test. At 95% confidence interval, p values less than 0.05 were considered to be significant.

Results

Effects of trazodone and its modulation by L-NAME, L-arginine and methylene blue on anxiety like behavior of sleep-deprived mice

Plus maze and Zero maze

Sleep deprivation for 72 hr significantly decreased average time spent per entry in open arm of plus maze performance task and zero maze as compared to naïve group (without sleep deprivation). Treatment with trazodone (5 and 10 mg/kg, ip) significantly increased the average time spent per entry in open arm in plus maze performance task and zero maze as compared to control (sleep deprived) ($p<0.05$) as shown in Table 1. L-NAME (selective nitric oxide synthase

inhibitors, 10 mg/kg, *per se*), methylene blue (non selective nitric oxide synthase inhibitors, 10 mg/kg, *per se*), pretreatment of the animals produced anti-anxiety like behavior in both the test models (Table 1). However, L-NAME (5 mg/kg), and methylene blue (10 mg/kg), pretreatments with trazodone (5 mg/kg) caused potentiation of antianxiety like effect as compared to their effect *per se* ($p<0.05$). There was increased average time spent per entry in open arm in both the test paradigm tasks. However, L-NAME (5 mg/kg) and methylene blue (10 mg/kg) *per se* did not produce any significant effect as compared to control ($p<0.05$). Further, L-arginine (nitric oxide precursor, 50 mg/kg) pretreatment with trazodone (5 mg/kg) reversed the protective effect of trazodone and reduced average time spent per entry in open arm as compared to trazodone (5 mg/kg) in both the test tasks ($p<0.05$). L-arginine (50 mg/kg) *per se* did not show any significant effect on anxiety like behavior in both the test models as compared to control ($p<0.05$).

Mirror chamber

The sleep deprivation significantly delayed the latency to enter in mirror chamber, decreased average time spent per entry in the mirror chamber as compared to the naïve group (without sleep deprivation). Treatment with trazodone (5 and 10 mg/kg, ip) for 5 days significantly shortened the latency to enter mirror chamber and increased average time spent per entry in mirror chamber as compared to control (sleep deprived) ($p<0.05$) as shown in Table 2. L-NAME (5 mg/kg) and methylene blue (10 mg/kg) pretreatment with trazodone (5 mg/kg) caused further improvement in their antianxiety effect (decreased latency to enter mirror chamber and increased average time

Table 1: Effect of trazodone and its modulation by L-NAME, L-arginine and methylene blue on plus maze performance task and zero maze test in 72-hr sleep deprived mice

Treatment (mg/kg)	Average time spent per entry in open arm (mean ± SEM)	
	Elevated plus maze test	Zero maze test
Naïve (without sleep deprived)	20.0 ± 1.29	21.5 ± 1.19
Control (Sleep-deprived)	7.0 ± 0.57[a]	7.0 ± 0.57[a]
Trazodone (5 mg/kg)	10.0 ± 1.9[b]	10.62 ± 1.01[b]
Trazodone (10 mg/kg)	13.27 ± 0.63[b,c]	14.37 ± 1.21 [b, c]
L-Arginine (50 mg/kg)	5.8 ± 2.18	6.0 ± 2.53
L-NAME(10 mg/kg)	7.25 ± 0.21	8.30 ± 1.71
Methylene blue (10 mg/kg)	7.75± 0.26	8.0 ± 0.38
L-NAME(10 mg/kg) + trazodone (5 mg/kg)	14.75± 0.9 [c,d]	13.62 ± 0.98 [c,d]
Methylene blue (10 mg/kg) +trazodone (5 mg/kg)	13.5± 0.48 [c,e]	13.75± 1.03 [c,e]
L-Arginine (50 mg/kg) + trazodone (5 mg/kg)	8.0 ± 0.83 [c]	8.25± 0.76 [c]

[a]p<0.05 as compared to naïve, [b]p<0.05 as compared to control (sleep deprived), [c]p<0.05 as compared to trazodone (5mg/kg), [d]p<0.05 as compared to L-NAME (10 mg/kg), [e]p<0.05 as compared to methylene blue (10 mg/kg)

Table 2: Effect of trazodone and its modulation by L-NAME, L-arginine and methylene blue on mirror chamber test in 72 hr sleep deprived mice

Drug Treatment	Latency to enter mirror chamber (mean ± SEM)	Average time spent per entry in mirror chamber (mean ± SEM)
Naïve (without sleep deprived)	117.25 ± 5.2	16.75 ± 1.65
Control (sleep-deprived)	210.3 ± 7.13[a]	6.3 ± 1.6[a]
Trazodone (5 mg/kg)	187.75 ± 3.2[b]	10.87 ± 0.7[b]
Trazodone (10 mg/kg)	137.7 ± 4.1[c]	13.87 ± 0.6[c]
L-NAME (10 mg/kg)	198.7 ± 7.2	7.25 ± 0.33
Methylene blue (10 mg/kg)	203.5 ± 4.6	7.75 ± 0.27
L-Arginine (50 mg/kg)	222.7 ± 5.1	4.8 ± 3.32
L-NAME (10 mg/kg)+ trazodone (5 mg/kg)	173.4 ± 2.8[c,d]	15.0 ± 0.5[c,d]
Methylene blue (10 mg/kg) + trazodone (5 mg/kg)	176.5 ± 4.6[c,e]	13.25± 1.51[c,e]
L-arginine (50 mg/kg) + trazodone (5 mg/kg)	236.75 ± 4.9[c]	7.7 ± 3.2[c]

[a]p<0.05 as compared to naïve, [b]p<0.05 as compared to control (sleep deprived), [c]p<0.05 as compared to trazodone (5mg/kg), [d]p<0.05 as compared to L-NAME (10 mg/kg), [e]p<0.05 as compared to methylene blue (10 mg/kg)

spent per entry in mirror chamber) which was significant as compared to their effect per se (p<0.05). L-NAME (10 mg/kg, *per se*) and methylene blue (10 mg/kg, *per se*) did not produce any significant effect on anxiety like behavior as compared to control (p<0.05). Further, pretreatment with L-arginine (50 mg/kg) with trazodone (5 mg/kg) reversed the antianxiety-like effect of trazodone. There was delayed latency to enter mirror chamber and reduced average time spent per entry in mirror chamber as compared to trazodone (5 mg/kg) (p<0.05). L-arginine (50 mg/kg per *se)* did not produce any significant effect on anxiety-like behavior as compared to control (p<0.05).

Effects of trazodone and its modulation by L-NAME, L-Arginine and methylene blue on locomotor activity of sleep-deprived mice

The effects of trazodone and its modulation by L-NAME, L-Arginine and methylene blue on locomotor activity of sleep-deprived mice are given in Table 3. The locomotor activity of the 72 hr sleep deprived mice were significantly reduced as compared to naïve mice. Treatment with trazodone (5 and 10 mg/kg, ip) for 5 days significantly improved locomotor activity (p<0.05). L-NAME (10 mg/kg) *per se* and methylene blue (10 mg/kg) *per se* and its pretreatment with trazodone (5 mg/kg) did not significantly influence the locomotor activity as compared to their own effect alone (p<0.05). Similarly, L-arginine (50 mg/kg*) per se* and its

Table 3: Effects of trazodone and its modulation by L-NAME, L-arginine and methylene blue on locomotor activity of sleep-deprived mice

Treatment	Counts in 5 min (mean ± SEM)
Naïve (without sleep deprived)	233 ±1.95
Control (sleep-deprived)	97.25 ±5.17 [a]
Trazodone (5 mg/kg)	141.75 ±2.01 [b]
Trazodone (10 mg/kg)	192.0 ±1.5 [c]
L-NAME (10)	107.75 ±5.8
Methylene blue (5 mg/kg)	149.75±6.3
Methylene blue (10 mg/kg)	107.25±6.6
L-Arginine (50 mg/kg)	92.5±5.1
L-NAME (10 mg/kg) + trazodone (5 mg/kg)	150.0±7.8
L-Arginine (50 mg/kg)+ trazodone (5 mg/kg)	137.0±6.6

[a]$p<0.05$ as compared to naïve, [b]$p<0.05$ as compared to control (sleep deprived), [c]$p<0.05$ as compared to trazodone (5mg/kg), [d]$p<0.05$ as compared to L-NAME (10 mg/kg), [e]$p<0.05$ as compared to L-arginine (50 mg/kg) and [f]$p<0.05$ as compared to methylene blue

combination with trazodone (5 mg/kg) did not influence locomotor activity as compared to their own effect alone.

Effects of trazodone and its modulation by L-NAME, L-arginine and methylene blue on oxidative stress parameters of sleep-deprived brain

The effects of trazodone and its modulation by L-NAME, L-arginine and methylene blue on oxidative stress parameters of sleep-deprived brain are shown in Table 4. The 72 hr sleep deprivation significantly increased lipid peroxidation, nitrite levels, depleted reduced glutathione level and catalase activity in the mice ($p<0.05$). Treatment with trazodone (5 and 10 mg/kg, ip) significantly restored reduced glutathione and catalase activity, attenuated elevated lipid peroxidation and nitrite concentration as compared to 72 hr sleep deprived mice ($p<0.05$). L-NAME (10 mg/kg) and methylene blue (10 mg/kg) pretreatment with trazodone (5 mg/kg) caused further significant potentiation in their antioxidant activity as compared to their effect *per se* ($p<0.05$). However, L-NAME (10 mg/kg) *per se* and methylene blue (10 mg/kg) *per se* treatment did not have any significant effect on the antioxidant level as compared to the control ($P<005$). Further, L-arginine (50 mg/kg) pretreatment with trazodone (5 mg/kg) significantly reversed the protective effect of trazodone (5 mg/kg) ($p<0.05$).

Discussion

Disturbance in sleep is a common problem[30] that requires appropriate diagnosis and management, whether it is due to anxiety, depression or a hectic lifestyle. A number of neuropsychiatric problems such as depression, anxiety, psychosis occur due to chronic sleep deprivation that impairs brain functions and contributes to allostatic load of the body. Several research reports suggest that sleep has an important role in motor activity, anxiety level, memory dysfunction, body weights and metabolic function, such as reduced anabolic hormones, etc[31,32]. However, the mechanism of these behavioral

Table 4: Effects of trazodone and its modulation by L-NAME, L-arginine and methylene blue on oxidative parameters of sleep-disturbed mice (percentage of control in parentheses)

Treatment (mg/kg)	Lipid peroxidation (moles of malondialdehyde /mg protein)	Glutathione (µmoles of Glutathione S-transferase/mg protein)	Catalase (µ mole of H_2O_2/min/mg protein)	Nitrite (µg/ml)
Naïve (without sleep deprived	0.118±0.005 (28.85)	0.03±0.002 (750)	2.65±0.26 (361.5)	162.5±4.9 (32.01)
Control (sleep-deprived)	0.409±0.003 [a] (100)	0.004±0.0008 [a] (100)	0.733±0.68 [a] (100)	507.5±4.28 [a] (100)
Trazodone (5 mg/kg)	0.297±0.001[b] (60.61)	0.012±0.001 [b] (300)	1.3±0.23 [b] (177.3)	433±3.9 [b] (85.3)
Trazodone (10 mg/kg)	0.197±0.003[c] (48.16)	0.022±0.001 [c] (550)	2.03±0.3 [c] (276.9)	315.75±4.6 [c] (62.21)
L-NAME (10 mg/kg)	0.354±0.001 [b] (0)	0.008±0.001 [b] (0)	0.999±0.19 [b] (0)	470.5±4.3 [b] (0)
L-Arginine (50 mg/kg)	0.429±0.003[b] (0)	0.003±0.001 (0)	0.650±0.02 [b] (0)	516.25±5.6 [b] (0)
Methylene (10 mg/kg)	0.369±0.002[b] (0)	0.007±0.002[b] (0)	0.980±0.33[b] (0)	483.25±3.7[b] (0)
L-NAME (10 mg/kg) + trazodone (5 mg/kg)	0.220±0.004[c,d] (53.7)	0.017±0.001 [c, d] (425)	1.53±0.61 [c, d] (208.7)	397±3.2 [c, d] (78.2)
Methylene (10 mg/kg) + trazodone (5 mg/kg)	0.226±0.003 [c, e] (55.25)	0.016±0.001 [c, e] (400)	1.59±0.37 [c, e] (216.9)	387.2±5.1 [c, e] (76.29)
L-Arginine (50 mg/kg) + trazodone (5 mg/kg)	0.347±0.002 [c, f] (84.84)	0.007±0.002 [c, f] (175)	0.88±0.53 [c, f] (120)	471.7±4.8 [c, f] (92.9)

[a]p<0.05 as compared to naïve, [b]p<0.05 as compared to control (sleep deprived), [c]p<0.05 as compared to trazodone (5mg/kg), [d]p<0.05 as compared to L-NAME (10 mg/kg), [e]p<0.05 as compared to methylene blue (10 mg/kg), [f]p<0.05 as compared to L-arginine (50 mg/kg).

and biochemical changes produced during sleep deprivation are still largely unknown or poorly understood.

In the present study, 72 hr sleep deprivation caused significant impairment in locomotor activity and anxiety-like behavior in animals. Silva et al[33] have also reported that sleep deprivation causes anxiety-like behavior and influence motor behavior. Increased cortisol level has been linked with anxiety-like behavior and decreased motor behavior response in humans[34]. Sleep deprivation significantly influence brain functions and

causes long-term changes in multiple neural systems. Marked behavioral changes might be due to alterations in the brain regions controlling motor activity and anxiety like behavior[33,35,36].

Trazodone is a well-known antidepressant drug used in the management of mild to moderate depression. Antidepressant drug treatment has also been reported to improve quality of sleep[37], sleep efficiency[37]. Besides, antidepressant drug treatment also improves motor activity[38], antianxiety[31,38], and antioxidative effect[39,40]. In our study, trazodone treatment significantly improved locomotor activity and produced antianxiety effect in all the behavior paradigms suggesting therapeutic potential of the drug in the management of behavioral alterations associated with sleep deprivation.

Recently, the role of oxidative stress has been suggested in sleep deprivation[41]. Reports suggest that sleep deprivation increase free radicals and weakens oxidative defense by altering the balance between oxidants and antioxidant defense[11,42,43]. Oxidative stress indicators (lipid peroxidation, nitrite level) and antioxidant defense parameters (glutathione, catalase level) may be useful to understand the oxidative damage cascades in sleep deprivation. However, little is known as to whether stress is an important consequence of sleep deprivation. In our study, 72-hr sleep deprivation caused significantly oxidative damage as indicated by raised lipid perooxidation, nitrite concentration. Free radicals are well known to initiate a cellular cascade, causing lipid peroxidation, DNA damage and cell death. Besides, 72 hr sleep deprivation also weakened the antioxidant defense as evidenced by depletion of reduced glutathione and catalase activity. This suggests that the sleep deprivation cause significant oxidative damage possibly by unbalancing oxidative and antioxidant defense mechanism. The brain is more sensitive to oxidative damage due to an abundant presence of polyunsaturated fatty acids, a deficient antioxidant defense and high rate of oxygen utilization due to higher metabolic rate[12,34]. Ramanthan et al[11] also reported a significant decrease in superoxide dismutase activity in hippocampus and brain stem of sleep-deprived rat.

Nitric oxide (NO), an intercellular messenger in the brain, plays a crucial role in various physiological and pathological processes[44,45]. It also modulates sleep-wake rhythm of living beings. However, its exact role in sleep deprivation is still not clear[46,47]. Any pharmacological manipulation of nitric oxide pathway may be considered as a novel therapeutic approach for the management of sleep and related stress disorder. NO is an important neuromodualtor which plays a key role in sleep homeostasis from the fact that administration of a NOS inhibitor suppresses sleep rebound occurring in response sleep deprivation[48]. In our study, pretreatment with L-arginine (nitric oxide precursor) with trazodone, caused reversal of anti-anxiety like behavior and antioxidant effect of trazodone suggesting the involvement of free radical that influence sleep deprivation-induced behavioral and biochemical alterations. Beside, it has been reported that nitric oxide is involved in the mechanism of anxiety[49,50]. Antidepressant drugs also acts by involving NO-cGMP pathway[51]. L-Arginine (NO precursor) caused anxiety like behavior in all the tests paradigm tasks in our study, indicating that NO may be an anxiogenic substance. However, its exact role in the neurobiology of anxiety is still unclear. NO reacts with reactive oxygen species and act as an oxidant agent. The oxidation is not specific and affects any cell molecule. Besides, NO reacts with reactive oxygen species quickly enough to avoid the action of antioxidant system, forming peroxynitrite anion ($ONOO^-$)[44,45]. However, the role of reactive oxygen species - reactive nitrogen species (ROS-RNS) and its interaction with NO in the regulation of stress

responses are, so far, not clearly understood. Further, reports indicate that systemic administration of L-NAME (selective NOS inhibitor) and methylene blue (non-selective NOS inhibitor) cause's anxiolytic and antioxidant effect[49,52]. Further, co-administration of L-NAME and methylene blue pretreatment with trazodone caused the potentiation of trazodone's protective effect against sleep deprivation induced behavioral alteration and oxidative damage, suggesting the involvement of nitric oxide pathway. However, both l-arginine and L-NAME and its pretreatment with trazodone did not produce any significant effect on locomotor activity of the animals, suggesting that nitric oxide is not significantly involved in these effects. Ou study further suggests the possibility that the inhibition of nitric oxide synthase pathway could be used as a strategy to enhance the clinical efficacy of trazodone.

Conclusion

The present study has shown that nitric oxide modulation is involved in the protective effect of trazodone. In addition, the protective effects of trazodone might be partially due to its antioxidant activity.

Acknowledgements

Authors are thankful to the financial support of Grants Commission (UGC), New Delhi, and University Institute of Pharmaceutical Sciences for providing infrastructure facility for carrying out this work.

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