Possible role of citalopram and desipramine against sleep deprivation-induced anxiety like-behavior alterations and oxidative damage in mice

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Received 11 February 2008; revised 4 September 2008

Sleep is an essential physiological process for maintaining physical, mental, and emotional health. Sleep deprivation and associated disorders like depression and anxiety are one of the major problems now-days. The present study was designed to explore the neuroprotective effect of citalopram and desipramine on 72 hr sleep deprivation-induced behavioral alterations and oxidative damage in mice. Various behavioral tests (plus maze, zero maze, mirror chamber, actophotometer), body weight followed by oxidative parameters (malondialdehyde level, glutathione, catalase, nitrite and protein) were assessed. Treatment with citalopram (5 and 10mg/kg, ip) and desipramine (10 and 20 mg/kg, ip) for 5 days significantly improved locomotor activity, anti-anxiety like behavior in all paradigms tasks (mirror chamber, plus maze, zero maze) as compared to control (72 hr sleep-deprived). Biochemically, citalopram and desipramine treatment significantly restored depleted reduced glutathione, catalase activity, attenuated raised lipid peroxidation and nitrite level as compared to control (72 hr sleep-deprived) animals. Results of present study suggest that citalopram (5 and 10mg/kg, ip) and desipramine (10 and 20 mg/kg, ip) have neuroprotective effect against sleep deprivation-induced behavior alteration and oxidative damage in mice

Keywords: Anxiety, Citalopram, Desipramine, Locomotor activity, Oxidative stress, Sleep deprivation

Sleep is a restorative process that regulates homeostasis of autonomic, neuroendocrine and immune systems. Sleep deprivation not only alters sleep-waking pattern, but in spite of increase in food intake results in reduction in body weight, initial hyperthermia followed by hypothermia, anxiety like behavior, impaired motor activity behavioral irritability and poor performance. Sleep deprivation results an increase in plasma glucocorticoids in both humans and rodents and leads to aging process due to increase brain cortisol level, which further causes neurodegeneration over a period of time. Chronic sleep disturbances aggravate health risk factors that appear to compound disease processes.

Brain tissues are rich in polyunsaturated fatty acids and are metabolically active. Sleep deprivation causes oxidative stress and impair antioxidative defense mechanism of the body. The antioxidative levels of brain tissues are low as compared to other tissues of the body i.e. it has low levels of the catalase enzyme activity.

Recent epidemiological studies strongly suggest that not only is insomnia a typical symptom of depression but vice-versa, insomnia may be an independent risk factor for depression in the long run. The relation between insomnia and depression constitutes a situation with evidence supporting a strong bi-directional linkage. More than 90% of depressed patients complain about impairments of sleep quality. Vollrath et al. from long-term epidemiology study in Switzerland reported that 25% of the patients complaining of chronic insomnia suffered from depression.

Citalopram and desipramine are well known drugs used to cure depression. Sleep deprivation often causes depression. Therefore, antidepressants are present to manage sleep deprivation and related problems. Studies have also suggested that up to 90% of patients suffering from major depressive disorder experience some type of sleep disruption. Disturbance of sleep is a common problem in depressed patients and antidepressant drugs have been reported to improve the quality of sleep in such patients. Therefore, regulation of sleep can be an essential component for understanding the pathophysiology and treatment of depression. It indicates that there must be significant overlap in
neurotransmitters system and possibly, the circuits that regulate sleep and mood. Depression-related sleep disturbances include difficulty in initiating and maintaining sleep, abnormal sleep architecture reduced slow wave sleep (SWS) time, and greater number of stage shifts (NSS) and rapid eye movement (REM) sleep deregulation. Besides, persistent sleep disturbances are also a risk factor to depression. Lower doses of antidepressants are frequently prescribed to manage insomniac patients without depression. Typical antidepressants like tricyclic antidepressants (TCAs), improve sleep continuity in such patients. Whereas a typical antidepressant like most selective serotonin reuptake inhibitors (SSRIs), may exhibit alerting effects, thereby reducing total sleep time and sleep efficiency, and increasing wakefulness. However, exact mechanism involved in treatment of depression caused due to sleep deprivation is not clear. The present study has been undertaken to study the possible role of antidepressants (citalopram and desipramine) against sleep deprivation-induced anxiety like-behavior alterations and oxidative damage in mice.

**Materials and Methods**

**Animals**—Albino mice (n= 36) (Laca strain) weighing between 22-30g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Animals were divided in to six groups, consist of minimal 6 animals each. All experiments were carried out between 0900 and 1500 hrs. 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 6 groups. Naïve (vehicle) and control (sleep deprived) were treated as group 1 and 2, citalopram (5 and 10mg/kg) and desipramine (10 and 20mg/kg) were treated as group 3 to 6 respectively. Citalopram and desipramine was freshly prepared in distilled water and administered, ip for 5 days, starting two days before 72 hr sleep deprivation.

**Sleep deprivation**—Animals were sleep deprived for 72 hr by placing them on grid suspended over water method as described by Sinomiya et al. Animals were placed on a grid floor (29×15×7 cm) inside the plastic cage filled with water to 1cm 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**— Various behavioral parameters were assessed in mice after 72 hr sleep deprivation-induced stress.

**Measurement of anxiety levels**

**Elevated plus maze test**—Elevated plus maze developed by Pellow is a novel test for testing selective anxiogenic and anxiolytic drug effect in rodents. The plus maze apparatus consist of two open (16 × 5 cm) and two closed arm (16 × 5×12 cm) and placed at a height of 25 cm from ground. The mice were placed individually at the center 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 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). Animals were placed in the closed quadrant. During the 5 min test, average time spent per entry in open arm of the maze the maze was recorded.

**Mirror chamber test**—The mirror chamber consists of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session (a) latency to enter the mirror chamber and (b) average time spent per entry in mirror chamber were noted. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased average time spent per entry in the mirror chamber.

**Measurement of locomotor activity**—The locomotor activity was recorded using actophotometer for a period of 5 min. The apparatus was placed in a darkened, light - sound attenuated and ventilated testing room. Each animal was observed for 5 min in
a square (30 cm) closed arena equipped with infrared light sensitive photocells using digital photoactometer and values expressed as counts/5 min.

Biochemical tests

Tissue preparation—All the 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 Wills31. The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as n mole of malondialdehyde/mg protein using the molar extinction coefficient of chromophore (1.56×10M⁻¹ cm⁻¹).

Estimation of reduced glutathione — Reduced glutathione in the brain was estimated according to the method of Ellman32. A 1.0 ml of homogenate was precipitated with 1.0 ml of 4% sulfoalicylic acid by keeping the mixture at 4°C for 1 hr and the samples were immediately centrifuged at 1200 g for 15 min at 4°C. The assay mixture contains 0.1ml of supernatant, 2.7 ml of phosphate buffer of (pH 8.0) and 0.2 ml of 0.01M dithiobisnitrobenzoic acid (DTNB). The yellow color developed was read immediately at 412 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as n mole GSH/mg protein.

Nitrite estimation—Nitrite is the stable product of nitric oxide (NO) in living system. Accumulation of nitrite was measured in cell free supernatants from brain homogenates by spectrophotometer assay based on Greiss reagent 15 (1% sulphanilamide 0.1% naphthylethlenediamine dihydrochloride 2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore. Absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as µmole nitrite/mg homogenate33.

Protein estimation — The protein content was measured according to the method of Lowry34 using bovine serum albumin as standard.

Catalase estimation—Catalase activity was assayed by the method of Luck35, wherein the breakdown of hydrogen peroxides (H₂O₂) is measured at 240 nm. Briefly, assay mixture consisted of 3 ml of H₂O₂ phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and change in absorbance was recorded at 240 nm. The results were expressed as µmole H₂O₂ decomposed per milligram of protein/min.

Statistical analysis—All the values are expressed as mean ± SE. The data were analyzed using analysis of variance (ANOVA) followed by Tukey test. In all the test criterion for statistical significance was P<0.05.

Results

Effect of citalopram and desipramine on anxiety like behavior in sleep-deprived mice: Plus maze and Zero maze test — 72 hr sleep deprivation significantly decreased average time spent per entry in open arm of both plus maze and zero maze as compared to naïve group (without sleep deprivation). Treatment with citalopram (5, 10 mg/kg) and desipramine (10 and 20 mg/kg) significantly increased average time spent per entry in open arm of both plus maze and zero maze as compared to control (sleep deprived) (P<0.05; Fig. 1).

Mirror chamber—72 hr sleep deprivation significantly increased latency to enter in mirror chamber, decreased average time spent per entry in the mirror chamber as compared to naïve group (without sleep deprivation). Treatment with citalopram (10 mg/kg) and desipramine (10 and 20 mg/kg) for 5 days significantly decreased time latency to enter in mirror chamber, increased average time spent per entry in mirror chamber as compared to control (sleep deprived)(P<0.05; Figs. 2 and 3).

Effect of citalopram and desipramine on locomotor activity of sleep-deprived mice — 72 hr sleep deprivation significantly reduced locomotor activity as compared to naïve mice (without sleep deprivation). Treatment with citalopram (5 and 10 mg/kg) and desipramine (10 and 20 mg/kg) for 5 days significantly improved locomotor activity (P<0.05; Fig. 4).

Effect of citalopram and desipramine on antioxidant parameters of sleep-deprived mice — 72 hr sleep deprivation significantly increased lipid peroxidation, nitrite levels and decreased glutathione level, catalase activity as compared to naïve (without sleep deprived) mice. Treatment with citalopram
(5 and 10 mg/kg) and desipramine (10 and 20 mg/kg) significantly restored depleted glutathione and catalase activity, attenuated elevated lipid peroxidation and nitrite activity as compared to the 72 hr sleep deprived mice ($P<0.05$; Table 1).

**Discussion**

Sleep has important homeostatic functions and sleep deprivation is a kind of stressor that influences many body systems. Disturbed sleep is a common problem now a days, requiring appropriate diagnosis and management, whether sleep deprivation is due to anxiety, depression, or a hectic lifestyle. There are consequences of chronic sleep deprivation that impair brain functions and contribute to allostatic load of the body. Sleep has an important role in motor activity, anxiety level, memory dysfunction, body weights and metabolic function such as reduced anabolic hormones etc. However, these behavioral and biochemical changes produced by sleep deprivation are still largely unknown or poorly understood. 

![Fig. 1](image1.png)

**Fig. 1**—— Effect of citalopram and desipramine on (A) plus maze performance task (B) zero maze task in 72 hr sleep deprived mice. [values are mean ± SE. $^aP$ values: <0.05 as compared to naïve, $^bP<0.05$ as compared to control (sleep deprived), $^cP<0.05$ as compared to citalopram (5 mg/kg), $^dP<0.05$ as compared to desipramine (10 mg/kg)]. Cit = citalopram, desi = desipramine.

![Fig. 2](image2.png)

**Fig. 2**—— Effect of citalopram and desipramine on latency in mirror chamber test in 72 hr sleep deprived mice. [Values are mean ± SE. $^aP$ values: <0.05 as compared to naïve, $^bP<0.05$ as compared to control (sleep deprived), $^cP<0.05$ as compared to citalopram (5 mg/kg), $^dP<0.05$ as compared to desipramine (10 mg/kg)].

![Fig. 3](image3.png)

**Fig. 3**—— Effect of citalopram and desipramine on average time per entry in mirror chamber test in 72 hr sleep deprived mice. [values are mean ± SE. $^aP$<0.05 as compared to naïve, $^bP<0.05$ as compared to control (sleep deprived), $^cP<0.05$ as compared to citalopram (5 mg/kg), $^dP<0.05$ as compared to desipramine (10 mg/kg)].

![Fig. 4](image4.png)

**Fig. 4**—— Effects of citalopram and desipramine on locomotors of sleep-deprived mice. [values are mean ± SE. $^aP$<0.05 as compared to naive, $^bP<0.05$ as compared to control (sleep deprived), $^cP<0.05$ as compared to citalopram (5 mg/kg), $^dP<0.05$ as compared to desipramine (10 mg/kg)].
Depressive illness frequently disturbs sleep-wake, and hormone secretion rhythms. In the present study, 72 hr sleep deprivation caused significant impairment in locomotor activity and severe anxiety-like behavior in animals. Sleep deprivation causes anxiety like behavior and influence motor behavior35. Marked behavioral changes may be due to alteration in the brain regions involved such as hippocampus, amygdala, and prefrontal cortex that undergo structural remodeling that aggravate memory dysfunction, anxiety and aggression35,36.

Citalopram (SSRI), and desipramine (TCA) are well-known antidepressants, used for the management of depression. Depression-related sleep disturbances also caused difficulty in initiating and maintaining sleep13. Several antidepressants have also been reported to improve quality of sleep continuity 20. Selective serotonin reuptake inhibitors (SSRI's) decrease total sleep time and sleep efficiency21. In the present study, 72hr sleep deprivation significantly altered sleep-wake cycle and impaired motor activity. Like depression, sleep deprivation also produces similar symptoms suggesting that there must be pathological link between sleep disorders and depression37,38. Antidepressant drug treatment has also been reported to improve quality of sleep and sleep efficiency39. Besides, antidepressant drug treatment also improves weight loss, locomotor activity, antianxiety41,42 and antioxidative effect43,44. Role of oxidative stress has been suggested in sleep deprivation45. Indeed, increase in hypothalamic and thalamic oxidative stress levels was found in sleep-deprived rats46. Ramanthan10 also reported a significant decrease in superoxide dismutase activity in hippocampus and brain stem in sleep-deprived rats. Oxidative stress indicators lipid peroxidation, glutathione, catalase and nitrite level may be useful to understand the oxidative damage cascades in sleep deprivation. However, little is known, whether stress is an important consequence of sleep deprivation.

In the present study, 72hr sleep deprivation significantly caused oxidative damage as indicated by raised level of aggressive factor (lipid peroxidation and nitrite level) and lowering of defense factors (reduced glutathione). Reimund47 proposed that sleep increases the efficiency of antioxidant mechanisms in the brain. Further, citalopram (5 and 10 mg/kg) and desipramine (10 and 20 mg/kg) treatment reversed the lipid peroxidation and nitrite level and restored depleted reduced glutathione and catalase activity in stressed brain, suggesting its antioxidant activity. This indicates that citalopram (5 and 10 mg/kg) and desipramine (10 and 20 mg/kg) could be used as neuroprotective in sleep deprivation-induced behavioral and biochemical changes.

Conclusion
The present study has shown that citalopram and desipramine produces neuroprotective effect against sleep deprivation-induced behavioral alterations and oxidative damage. Present findings further support the therapeutic potential of citalopram and desipramine as neuroprotectant in the treatment of sleep deprivation-related oxidative damage.

References
1 Berger R J, Bioenergetic functions of sleep and activity rhythms and their possible relevance to aging, Fed Proc; 34 (1975) 97.

Table 1—Effects of citalopram and desipramine on oxidative parameters of sleep-disturbed mice

<table>
<thead>
<tr>
<th>Drug treatment (mg/kg)</th>
<th>LPO (moles of MDA/mgpr)</th>
<th>Nitrite (µg/ml)</th>
<th>Catalase (µ Mole of H2O2/min/mgpr)</th>
<th>GSH (micromoles of GSH/mgpr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>0.118±0.005</td>
<td>162.5±4.78</td>
<td>2.65±0.263</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Control</td>
<td>0.409±0.003</td>
<td>507.5±7.5</td>
<td>0.733±0.68</td>
<td>0.004±0.0008</td>
</tr>
<tr>
<td>Citalopram (5)</td>
<td>0.277±0.003b</td>
<td>382.5±8.53</td>
<td>1.62±0.005b</td>
<td>0.013±0.002b</td>
</tr>
<tr>
<td>Citalopram (10)</td>
<td>0.178±0.003c</td>
<td>200±9.12</td>
<td>2.02±0.131c</td>
<td>0.025±0.001c</td>
</tr>
<tr>
<td>Desipramine (10)</td>
<td>0.208±0.006d</td>
<td>290±10.8</td>
<td>1.81±0.122d</td>
<td>0.022±0.0007d</td>
</tr>
<tr>
<td>Desipramine (20)</td>
<td>0.208±0.006</td>
<td>290±10.8</td>
<td>1.81±0.122d</td>
<td>0.022±0.0007d</td>
</tr>
</tbody>
</table>

*p values: <0.05 as compared to naïve, †P<0.05 as compared to control (sleep deprived), ‡P<0.05 as compared to citalopram (5 mg/kg), ‡P<0.05 as compared to desipramine (10 mg/kg).


