Effect of Green Tea Extract (Camellia Sinensis) On Spatial Learning And Memory In Rem Sleep Deprived Albino Wistar Rats

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ABSTRACT

Introduction: Sleep deprivation can enhance the metabolic rate and oxidative stress, causing memory deficits. Green tea (Camellia sinensis), has shown to have radical scavenging, gene modulating, and cell signalling activities rendering it to have neuroprotective action.

Objective: To evaluate the possible beneficial effect of aqueous extract of green tea (GTE) on spatial learning and memory in rapid eye movement (REM) sleep deprived rats.

Methodology: Wistar albino rats were deprived of REM sleep for 96 h using the modified inverted flowerpot technique. Morris water maze, antioxidants levels and body weight were used to assess cognitive enhancing and metabolic activity.

Results: Increase in oxidative stress following REM sleep deprivation was reversed in both acute and chronic study. Chronic intake of GTE mitigated spatial learning and memory deficit in Wistar rats induced by REM sleep deprivation.

Conclusion: Oxidative stress is associated with memory deficits induced by sleep deprivation in the present study.

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INTRODUCTION
Sleep plays a pivotal role in normal biological functions, thought to be a process that facilitates neuronal and synaptic plasticity, which in turn is crucial for brain function and cognitive performance. It has been shown that REM stage of the sleep cycle is essential for spatial learning and memory by enhancing hippocampal-dependent memory consolidation. Disruption of REM sleep can disrupt other physiological systems resulting in hyperphagia and weight loss also. Numerous animal studies have demonstrated that REM sleep deprivation produces memory deficits. The mechanisms responsible for memory deficits following sleep deprivation is not clearly understood. A recent theory states that sleep promotes the endogenous anti-oxidant mechanism activities and decreases the production of free radicals in the brain. Thus sleep essentially plays the role of a facilitator of antioxidants. Additionally, Zepelin and Rechtschaffen believe that sleep limits metabolic requirements. Therefore, sleep deprivation can increase metabolic rate and oxidative stress as well.

Tea, from the plant Camellia sinensis, are most popular beverages consumed worldwide over the centuries, in its various forms including green, black, or oolong tea. Green tea is a ‘non-fermented’ tea. The potentially active medicinal principle in green tea is catechins. Green tea contains more catechins, than other forms of tea. Major catechins present in green tea are (a) epicatechin, (b) epicatechin-3-gallate, (c) epigallocatechin (EGC), and (d) epigallocatechin-3-gallate (EGCG). EGCG being the most abundant constituent accounting for 65% of the total catechin content and is probably responsible for most of its medicinal properties. Catechin in combination with theanine has been revealed to be neuroprotective in ischemia-reperfusion insult of the brain. Caffeine is long recognized as a stimulant for the brain; it has been shown to enhance memory consolidation and memory retrieval and may alleviate neuron damage in Parkinson’s disease. The radical scavenging, gene modulating, and cell signalling activities combined with its ability to cross the blood-brain barrier, renders green tea extract protection on neurological insults. Hence, this study is being conducted to explore the possible effects of aqueous extract of Camellia sinensis on spatial memory and learning in REM sleep deprived rats.

MATERIALS AND METHODS
A total of 48 young adult male Wistar albino rats (Rattus norvegicus), around four months old, each weighing 150-250 grams were obtained from the Central Animal Research Facility of Manipal University, Manipal. Male rats were chosen in order to avoid bias as previous studies proved sex differences in rats’ performance in the Morris water maze. All rats were housed in the Central Animal House, Manipal, at room temperature (23 ± 2°C) with reversal 12 hrs light: dark environment, as rats are preferably nocturnal animals, their light-dark cycle is often reversed to enable experiments during their active, dark phase, without causing substantial sleep deprivation and night shifts for the experimenters. They were given standard laboratory feed (VRK Nutritional Solutions, Pune, India) and water ad libitum.

The animals were divided into two groups of 24 each, for the acute and chronic study. The 24 rats were further subdivided into four groups of 6 rats each. Group I as control animals, Group II as REM sleep deprived animals, Group III as Control animals treated with GTE and Group IV as REM sleep deprived animals treated GTE.

Preparation of aqueous extract of Camellia sinensis:
The green tea extract was made by soaking 10 g of loose green tea powder in 100 mL of hot deionized water (just at the brink of boiling), steeped for 3 minutes and then filtered. This solution contains approximately 200 mg of catechin. For acute study 20 mg/kg of catechin of green tea extract was given orally one hour before Morris Water Maze test and for chronic study 20 mg/kg of catechin of green tea extract was given orally...
extract was given orally daily for 8 weeks before Morris Water Maze test\textsuperscript{[13]}.  

**Sleep deprivation procedure**  
The set up used in this study was a modified multi-platform technique \textsuperscript{[13]}. The container consisted of rectangular perspex boxes (24 cm wide, 40 cm long and 30 cm deep) with a central 14 cm high, 6.5 cm diameter platform with surface area of 33 cm\textsuperscript{2}. The bottom of the cage was filled with 22 \textsuperscript{4}C water to within 2 cm of the top of the platform. The wire mesh lid of the cage held food. Sleep deprivation was induced in male Wistar rats by housing them on small platforms over water, during onset of sleep, it touched the water by tilting its head downwards or would fall into the water due to muscle atonia. Controls were housed in tanks with large platforms which had a surface area of 320 cm\textsuperscript{2}. The water in the cages was changed daily. All rats had free access to food and water and were weighed daily. The room was maintained at 23\textdegree{}C. Every day, for 4 days, rats were tested between 1000 h and 1300 h, in a place-learning set paradigm using a Morris water maze. Weight: platform area ratios were approximately 10:1 for sleep deprived (SD) rats and 1:1 for control rats.  

**Assessment of spatial learning and memory**  
MWM test is one of the gold standards for a test of spatial learning and memory \textsuperscript{[14,15]}. The container in the MWM was filled with water and maintained at 25\textdegree{}C. The water was made opaque by adding milk and divided into four quadrants (Q1, Q2, Q3, and Q4). A white platform (10 cm\textsuperscript{2}) placed in the centre of the target quadrant (Q4) of the pool was submerged approximately 2 cm below the surface of water. A black and white board was placed near the maze to provide cues for allowing the rats to develop a spatial map strategy. The placement of the platform and cues were kept constant during the training session. The water maze test consisted of two phases.  

**Spatial task acquisition phase:** The rats were trained between 10:00 and 13:00 in the water maze on 3 consecutive days. Each animal had to undergo four consecutive acquisition trials per day at an interval of 5 minutes, during which the rats were allowed to locate the hidden platform and remain there for 20 seconds. In each trial, the rat was released facing the wall of the maze from different positions around the perimeter of the tank (northeast Q1, northwest Q2, southeast Q3 and southwest Q4), respectively. If the animal was not able to reach the hidden platform, it was gently guided to the platform and allowed to remain there for 20 seconds. During each trial, the time taken by the rats to locate the hidden platform were noted.  

**Spatial probe trial:** On the 4th day following the completion of learning sessions, each rat was subjected to a spatial probe trial, in which no platform was placed. Each rat was placed in the water as mentioned in the acquisition phase and was allowed to explore the pool for 60 seconds. The parameters used to measure spatial working memory, the latency to enter and the total time spent in Q4 (target quadrant) were noted.  

**Biochemical estimations**  
**Collection of tissue samples:**  
The animals were sacrificed by cervical dislocation and brain tissues were carefully removed at the end of 96 h REM sleep deprivation in both acute and chronic study. Brain tissue was chilled in ice-cold phosphate buffer. After washing in ice-cold buffer, the brain was homogenized in phosphate phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer. The tissue homogenate was then utilized for malondialdehyde (MDA) assay and reduced glutathione levels assay \textsuperscript{[16]}.  

**RESULTS:**  
**Acute dose study**  
**Effect on bodyweight of animals**  
There was no statistically significant difference in the weight of animals between the groups, at the baseline in both acute and chronic dosing. At the end of 96 h sleep deprivation the mean weight of the sleep deprived rats increased (203±4.17) when compared to the control group (199.67±3.18) but was not statistically significant.
Effect on antioxidant levels in serum and brain tissue:
At the end of the REM sleep deprivation, sleep deprived control rats (group II) showed statistically significant (p<0.001) increase in MDA levels and decrease in GSH levels when compared with group I suggestive of oxidative stress in both serum as well as brain tissue. Group III showed statistically significant (p<0.001) increase in GSH level when compared with group I proving GTE as an antioxidant. Sleep deprived rats treated with green tea extract (group IV) showed statistical significant (p<0.001) decrease in MDA levels and increase in GSH levels when compared with group II suggestive of decrease in oxidative stress. Results are tabulated in table 1.

**TABLE 1: Serum and tissue antioxidant levels (MDA in μmol/g and GSH in μmol/mg protein)**  

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Post REM SD Serum MDA (Mean±S.E.M.)</th>
<th>Post REM Serum GSH (Mean±S.E.M.)</th>
<th>Tissue MDA (Mean±S.E.M.)</th>
<th>Tissue GSH (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.86±0.10</td>
<td>41.75±0.21</td>
<td>19.07±0.09</td>
<td>73.98±0.46</td>
</tr>
<tr>
<td>II</td>
<td>5.32±0.16(^a)</td>
<td>26.31±1.53(^a)</td>
<td>24.03±0.11(^a)</td>
<td>55.41±0.74(^a)</td>
</tr>
<tr>
<td>III</td>
<td>2.40±0.03</td>
<td>47.42±0.63(^b)</td>
<td>18.42±0.12</td>
<td>77.52±0.60(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>4.50±0.27(^d)</td>
<td>36.72±0.30(^d)</td>
<td>23.17±0.39</td>
<td>67.20±1.14(^d)</td>
</tr>
</tbody>
</table>

**Morris Water Maze Test**
Spatial memory during the acquisition trials

Post REM Sleep Deprivation
There was no statistically significant difference between the groups with respect to learning assessed by escape latency time on day one of learning. On the last day of learning, group IV showed statistical significant (p<0.01) increase in escape latency time when compared with group III. Results are shown in table 2.

**TABLE 2: Effect of GTE in REM sleep deprived rats during the acquisition trials on Day 1 and Day 3 of MWM**

<table>
<thead>
<tr>
<th>GROUPS (n=6)</th>
<th>Learning on Day 1 (Mean±S.E.M.)</th>
<th>Learning on Day 3 (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33.70±2.51</td>
<td>21.01±2.73</td>
</tr>
<tr>
<td>II</td>
<td>25.30±3.41</td>
<td>26.96±4.36</td>
</tr>
<tr>
<td>III</td>
<td>36.30±2.83</td>
<td>14.03±2.53</td>
</tr>
<tr>
<td>IV</td>
<td>31.25±1.62</td>
<td>33.83±3.16(^a)</td>
</tr>
</tbody>
</table>
Spatial probe trial Post REM Sleep Deprivation
Sleep deprived rats (group II) showed statistically significant (p<0.001) decrease when compared with group I. GTE treated normal rats showed statistically significant (p<0.001) increase in percentage time spent in the target quadrant when compared with group II and group IV. There was no statistical significant (p>0.05) difference between group II and group IV. Results are shown in table 3.

**TABLE 3: Effect of GTE during the probe trial of MWM test at the end of REM Sleep Deprivation**

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Total time spent in target quadrant (sec) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32.25±1.30</td>
</tr>
<tr>
<td>II</td>
<td>17.06±0.82*</td>
</tr>
<tr>
<td>III</td>
<td>33.10±1.52</td>
</tr>
<tr>
<td>IV</td>
<td>23.82±1.94*</td>
</tr>
</tbody>
</table>

[^1]p (0.001): group II, 4 vs group I; P value calculated by one-way ANOVA followed by Tukey’s post-hoc test

**CHRONIC DOSING**

i) Effect on body weight:
At the end of 96 h sleep deprivation, there was a statistically significant (p<0.001) difference in body weight of sleep deprived group II (223.17±6.06) and IV (220.17±6.12) when compared with group III (180.17±0.78) in chronic dosing of GTE.

ii) Effect on serum and brain tissue antioxidant levels:
After two months of dosing and at the end of 96 h REM sleep deprivation, group II showed statistically significant (p<0.001) increase in MDA levels and decrease in GSH levels when compared with group I, suggesting increase in oxidative stress. Group III showed statistical significant (p<0.001) decrease in MDA and increase in GSH levels when compared with group I. Group IV showed statistical significant (p<0.001) increase in GSH levels and decrease in MDA levels when compared to group II suggesting reversal of oxidative stress. Results are tabulated in table 4. The result were similar for serum and brain tissue antioxidants. This suggests that GTE reverses the oxidative stress induced by sleep deprivation.

**TABLE 4: serum and tissue antioxidant Levels [MDA in µmol/g and GSH in µmol/mg protein]**

<table>
<thead>
<tr>
<th>GROUPS (n=6)</th>
<th>Post REM SD MDA (Mean±S.E.M.)</th>
<th>Post REM SD GSH (Mean±S.E.M.)</th>
<th>Tissue MDA (Mean±S.E.M.)</th>
<th>Tissue GSH (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.7±0.06</td>
<td>42.26±0.52</td>
<td>18.77±0.09</td>
<td>73.43±0.53</td>
</tr>
<tr>
<td>II</td>
<td>4.92±0.09*</td>
<td>26.85±0.76a</td>
<td>23.03±0.19a</td>
<td>58.53±0.69a</td>
</tr>
<tr>
<td>III</td>
<td>1.70±0.05b</td>
<td>60.41±1.13b</td>
<td>15.48±0.11b</td>
<td>92.33±1.04b</td>
</tr>
<tr>
<td>IV</td>
<td>3.88±0.07c</td>
<td>45.25±0.58c</td>
<td>19.12±0.11c</td>
<td>83.95±0.64c</td>
</tr>
</tbody>
</table>

[^1]p <0.001 : group III vs group I;[^2]p <0.001 : group II vs group I;[^3]p <0.001 : group IV vs group II; P value calculated by one-way ANOVA followed by Tukey’s post-hoc test
Morris Water Maze Test
Spatial memory during the acquisition trials
Post REM Sleep Deprivation:

On day one of learning, group III statistical significant (p<0.001) decrease in escape latency time when compared with groups I. At the end of day three of learning, group II showed statistical significant (p<0.001) increase in escape latency time when compared with group I. Group III showed statistical significant (p<0.001) decrease in escape latency time when compared with group I. Group IV showed statistical significant (p<0.001) decrease in escape latency time when compared with group II. Results are shown in table 5.

TABLE 5: Effect of GTE in REM sleep deprived rats during the acquisition trials on Day 1 and Day 3 of MWM test.

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Learning Day 1 Escape Latency (Mean±S.E.M.)</th>
<th>Learning – Day 3 Escape Latency (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38.68±3.2</td>
<td>28.81±1.54</td>
</tr>
<tr>
<td>II</td>
<td>45.09±2.4</td>
<td>36.06±1.07</td>
</tr>
<tr>
<td>III</td>
<td>27.03±1.41a</td>
<td>11.96±1.12c</td>
</tr>
<tr>
<td>IV</td>
<td>36.19±2.66</td>
<td>21.52±0.79</td>
</tr>
</tbody>
</table>

[Escape latency in seconds]; [a p <0.001: group III vs group I; b p <0.001: group II vs group I; c p <0.001: group III vs group I; d p <0.001: group IV vs group II; P value calculated by one-way ANOVA followed by Tukey’s post-hoc test]

Spatial probe trial Post REM Sleep Deprivation

With respect to percentage time spent in the target quadrant, GTE treated normal rats (Group III) shows statistical significant (p<0.001) increase when compared with group I. Group II showed statistical significant (p<0.001) decrease when compared with group I. Group IV showed statistical significant (p<0.001) increase when compared with group II. Results are shown in table 6.

TABLE 6: Effect of GTE during the probe trial of MWM test at the end of REM Sleep Deprivation

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Time Spent In Target Quadrant (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32.31±1.78</td>
</tr>
<tr>
<td>II</td>
<td>19.52±1.02a</td>
</tr>
<tr>
<td>III</td>
<td>47.35±1.48</td>
</tr>
<tr>
<td>IV</td>
<td>25.70±0.99b</td>
</tr>
</tbody>
</table>

[a p (<0.001): group II vs group I; b p (<0.001): group IV vs group II; P value calculated by one-way ANOVA followed by Tukey’s post-hoc test]

DISCUSSION

In the present study, *Camelia sinensis* mitigated the spatial memory impairment induced by 96 h of REMSD in rats with chronic dosing. The data showed that the spatial learning and memory were impaired by 96 h REM SD. Increase in oxidative stress in the brain following REM sleep deprivation was also noted in the current study and this was also reversed using green tea extract.

In the current study, sleep-deprivation was induced in rats using the modified multiple platform model, which depends on the loss of muscle tone during REM sleep. This model produces a marked decrease of 90% to 95% in rapid eye movement (REM) sleep and this effect has been confirmed by Machado et al.,
2004 and Medeiros al., 1998 by using electroencephalographic recording to monitor sleep-deprivation [16]. Our data showed that acute REM sleep deprivation (96 h) impaired spatial learning and memory. Considerable studies have reached a consensus that REM sleep is closely related with cognition, and REM sleep deprivation impairs cognitive function. Previous studies have reported that continuous 72 h REM SD with the flowerpot method administered to rats during their training period was shown to impair both the acquisition rate in the Morris water maze and the ability to remember the position of the platform in the subsequent probe test [15]. Ruskin et al., also showed a decrease in learning by using modified version of the MWM after 72 h of REM SD administered before testing [18].

There was severe impairment of spatial reference memory performance with no deficit of spatial working memory in animals repeatedly tested over a 4–5-day REM SD period in previous studies [19]. The modified multiple platform models, which is used in the current study has a number of advantages over the other models of sleep deprivation. For instance, several animals can be deprived simultaneously, without laborious monitoring of electrophysiological characteristics of sleep. It also eliminates immobilization and isolation stress that occurs in the single platform model. However, it can be still affected by confounding factors, namely, stress and anxiety. It is notable that all models of sleep deprivation affected both REM and NREM phases of sleep to different degrees [13].

Sleep seems to limit metabolic requirements. Therefore, sleep deprivation could enhance metabolic rate and in turn increase the oxidative stress. The current study shows increase in MDA and decrease in total GSH following REM sleep deprivation suggesting free radical generation. Our data corroborate with previous reports that sleep deprivation induces hippocampal oxidative stress, which reflects on neuronal excitability, molecular signalling, and cognitive functions [19,20]. Mallic K et al have shown that REM sleep deprivation decreases membrane fluidity in the rat brain [21]. The study showed the oxidative damages observed in hippocampus, can contribute to the impairment of learning function [21].

The current study did not show any significant change in body weight in both acute as well as chronic study of GTE after 96 h REM sleep deprivation, in control group and also in GTE treated groups. Our study results were not in accordance with previously published reports. Very few studies including Bhanot JL et al., Mavanji V et al., showed that REM sleep deprivation increased intake of carbohydrate rich food and body weight gain in rats [22,23] but majority studies showed that sleep deprivation method resulted in weight loss including in human population [24,25].

In current study, acute dosing of GTE post REM sleep deprivation showed decrease in MDA level and increase in GSH both tissue and plasma. Similar results were obtained with chronic model after eight weeks of dosing with respect to antioxidant status in both brain tissue and plasma. These results are in accordance with previous reports where in catechins present in GTE could have a direct (antioxidant) or indirect (increase of activity or expression) effect in reducing oxidative stress [26,27,28,29].

Only chronic dosing of GTE attenuated spatial acquisition rate and subsequent retrieval impairment induced by 96 h REMSD in rats but not in acute study. Considerable studies have shown consistent results with the current study. Previous studies have demonstrated that green tea extract was given orally for a period of eight weeks improved spatial learning and memory in old male rats [30,31] and green tea catechin given for 26 weeks had an improved spatial working memory by modulating oxidative stress [32]. Green tea showed reversal of oxidative stress and improved spatial working memory in a streptozotocin-induced model of dementia [33,35]. It has been showed chronic intake of epigallocatechin-3-gallate ameliorates learning and memory deficits in diabetic rats via oxidative stress and also in stress-induced
impairment of learning and memory in rats [35,36].

CONCLUSION
In conclusion, the results of the present study indicate that acute intake of green tea extract failed to attenuate spatial learning and memory ability in 96 h REM sleep deprivation in experimental animals by reducing brain oxidative stress. But after chronic intake, green tea extract modulated oxidative stress and mitigates spatial learning and memory ability in male albino Wister rats induced by 96 h REM sleep deprivation. Considering the fact that involvement of oxidative stress in memory deficits induced by sleep deprivation in our current study, we speculate that cognitive impairment increased brain oxidative stress was associated with sleep deprivation. Further clinical studies are required to confirm these results.

REFERENCES:
17. Youngblood BD, Zhou J, Smagin GN, Ryan DH, Harris RB. Sleep deprivation by the “flower pot” technique and spatial


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