



## EFFICACY OF DONEPEZIL AND GALANTAMINE IN RETROGRADE AMNESIA

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### ABSTRACT

There has been an extensive study to identify how the treatment parameters of Electroconvulsive therapy (ECT) impinge on cognitive side effects. The present study was done to assess the efficacy of Donepezil and Galantamine on retrograde amnesia induced by ECT and is compared with Celecoxib as an in vivo probe. Male Wistar Rats were treated with Celecoxib, Donepezil 1mg/kg, 3 mg/kg and vehicle p.o respectively for 24 days, and administered with true and sham ECS on 21-23 day. Animals were treated with Galantamine 1mg/kg, 4mg/kg p.o for 15 days and administered with true and sham ECS on 12-14 day. Final transfer latency (TL) recorded using Elevated plus maze (EPM.) on the last day of treatment. For scopolamine induced amnesia, animals were treated with Donepezil 1mg/kg, 3 mg/kg, p.o., Galantamine 1mg/kg, 4 mg/kg and vehicle, p.o. for 8 days. On 9th day, Scopolamine(0.5 mg/kg, i.p) was injected and final TL was recorded using EPM. Donepezil 3mg/kg, Galantamine 4 mg/kg attenuated ECS induced amnesia by decreasing the final TL compared to True ECS vehicle control. Donepezil 3mg/kg, 1 mg/kg, Galantamine 1 mg/kg, 4mg/kg attenuated scopolamine induced amnesia by decreasing the final TL when compared with scopolamine control. Donepezil and Galantamine have the potential as an anti-amnesic agent against ECT induced retrograde amnesia.

**Keywords:** Acetylcholine, Donepezil, Galantamine, Electroconvulsive Shock, Elevated plus maze, Scopolamine.

### INTRODUCTION

Electroconvulsive therapy (ECT) is one of the most effective and safest available treatments for depression, ECT is also found to be beneficial in mania, schizophrenia, catatonia and other neuropsychiatric conditions. However, is associated with memory and non- memory forms of neuropsychological dysfunction. Memory deficits with ECT include impaired recall (retrograde amnesia) and impaired new learning (anterograde amnesia).<sup>1</sup> ECS down regulate muscarinic cholinergic receptors. This effect, therefore, may at least in part, explain the cognitive deficits associated with the treatment.<sup>2,3</sup>

Donepezil [(R,S)-1-benzyl-4[(5,6 dimethoxy-1-indanon)- 2-yl]-methyl piperidine hydrochloride] is a potent acetyl cholinesterase inhibitor<sup>4</sup> and Galantamine an allosteric enhancer of human  $\alpha 4$ ,  $\beta 2$  neuronal nicotinic receptor activity, human  $\alpha 7$  neuronal nicotinic acetylcholine receptor activity and muscuranic acetylcholine receptors and site of spontaneous cholinergic synaptic events.<sup>5</sup> Acetyl cholinesterase inhibitors inhibit the hydrolysis of acetylcholine and elevate its concentration in the synaptic cleft, provoking an increase of the efficacy of Cholinergic neurotransmission. Donepezil raises brain acetylcholine concentration as revealed by in vivo microdialysis studies in rats.<sup>4</sup>

In the present study, we sought to explore anti-amnesic activity of Donepezil and Galantamine using celecoxib as in vivo probe. Cholinergic facilitation may be involved in the memory impairment induced by ECT, hence the validity of the activity was envisaged using muscarinic receptor antagonist scopolamine, which induced amnesia.

### MATERIALS AND METHODS

#### Drugs, Chemicals and Equipments

Donepezil Hydrochloride from Donecept 10mg tablets (CIPLA), Celecoxib from Zycl 100 mg (Zydus healthcare), Scopolamine hydrobromide trihydrate(Sigma, CAS Number: 6533-68), Galantamine from Galamer 15mg tablets (Sun pharma) were used in the present study. ECT machine (Niviqure ECT device, Bangalore, India), Elevated plus maze and Actophotometer (INCO, Ambala, India) were also used.

#### Animal

Male Wistar rats weighing  $150 \pm 25$  gm, procured from Raghavendra Enterprises, Vijayanagar, Bangalore, were maintained under standard laboratory conditions of temperature  $25^{\circ} \pm 2^{\circ}$  C,  $70 \pm 5\%$  relative humidity and a natural day and night cycle. Commercial pellet diet (Amrut brand) supplied by pranav agro industries ltd. animal feeds, Bangalore) twice per day and water *ad libitum* were provided to the animals. The rats were divided into 12 groups of 8

animals each. Animals in group I, II, III, IV were treated with vehicle, Donepezil 1mg/kg and 3 mg/kg P.O. and Celecoxib 15mg/kg P.O. respectively for 24 days and grouped for true ECS. Animals in group V, VI, VII, VIII were treated as above, for sham ECS. Animals of group IX, X, were treated with Galantamine 1mg/kg and 4mg/kg P. O, respectively, for a period of fifteen days, for true ECS and same for animals of group XI, XII for sham ECS. Institutional Animal Ethics Committee's permission was obtained before starting the experimentation.

### EXPERIMENTAL

#### Administration of ECS

True ECS was administered for 3 days through gel-coated ear clip electrodes using the constant current, brief-pulse Niviqure ECT. (0.8 A pulse amplitude, 1.5 ms pulse width, 62.5 Hz pulse frequency bidirectional square waves administered in a stimulus train 0.2 s long.) The delivered charge was 30 mC. The duration of the ECS seizures was measured. The motor seizure was studied from the commencement of passage of current to the cessation of motor activity or the onset of asymmetrical limb movements, whichever occurred earlier. Sham ECS involved an identical procedure without passage of current<sup>6</sup>.

#### Scopolamine induced amnesia

For scopolamine induced amnesia, animals were divided into 6 groups of 8 animals each. Groups Ia, IIa, IIIa, IVa, Va were administered with Saline, Donepezil 1mg/kg, 3mg/kg i.p., Galantamine 1mg/kg, 4mg/kg i.p. respectively for 8 days. On the 9<sup>th</sup> day, 10 minutes after drug treatment, scopolamine 0.5 mg/kg was administered intraperitoneally to the animals of groups IIa, IIIa, IVa, Va and VIa. Transfer latency was noted 30 min there after.<sup>7</sup>

#### Elevated plus maze

Rats were placed individually at the end of open arm and the time taken to move to either of the closed arm (Transfer latency, TL) was recorded.<sup>8</sup> For ECS induced amnesia, Donepezil treated rats were allowed to explore the plus maze for the measurement of baseline TL on the days 20,21. ECS was administered on days 22-24. Final TL measured on day 25. In case of Galantamine treated rats, on day 9-11 the rats was allowed to explore the plus maze. Final TL was measured on day 15. For scopolamine induced amnesia, drugs were administered for 8 days, baseline TL recorded on 8<sup>th</sup> day and final TL was recorded on 9<sup>th</sup> day.

#### Locomotor Activity

The animals were individually placed in the Actophotometer cage

(30 × 25 × 25 cm) and the total activity count was registered for 300 seconds. The locomotor activity was expressed in terms of total cut off counts/300 sec per animal.<sup>9,10</sup>

### Statistical Analysis

The results are expressed as mean±SEM. Statistical analysis of ECS induced amnesia -elevated plus maze was done by one –way ANOVA, using SPSS (Statistical Package for the Social Sciences) and RMANOVA was used to analyze seizure duration. Statistical analysis for Scopolamine induced amnesia-elevated plus maze and closed field activities were done by One-Way ANOVA followed by Tukey Kramer's test. P<0.05 was considered to be statistically significant.

## RESULTS

### ECS induced amnesia

As shown in table 1 and Figure 1, ECS produced significant amnesia in the vehicle group. Donepezil 3mg/kg, Galantamine 4 mg/kg and

the standard drug Celecoxib 15 mg/kg significantly attenuated ECS-induced retrograde amnesia.

### Seizure duration

As shown in Figure 2, Seizure duration was significantly attenuated across time. Donepezil 1 mg/kg, 3 mg/kg, Celecoxib 15 mg/kg and vehicle had no effect on seizure duration.

### Scopolamine induced amnesia

As seen from table 2, Donepezil 3mg/kg, 1mg/kg and Galantamine 4mg/kg, 1mg/kg treated rats showed significant reduction in final TL on 9th day.

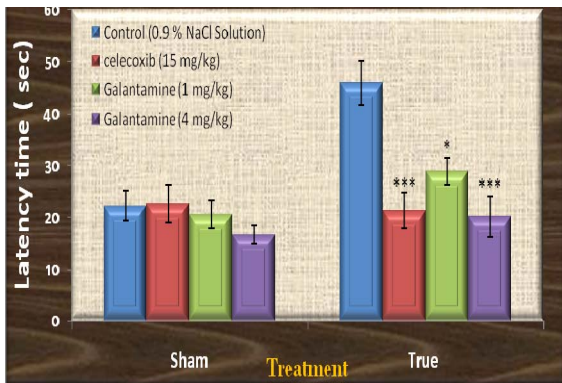
### Locomotor activity

Table 3 shows that the spontaneous locomotor activity in the vehicle, control, Donepezil 3mg/kg and 1mg/kg, Galantamine 4mg/kg, and 1mg/kg treated rats did not differ significantly.

**Table 1: Effect of Donepezil on final transfer latency on ECS induced amnesia.**

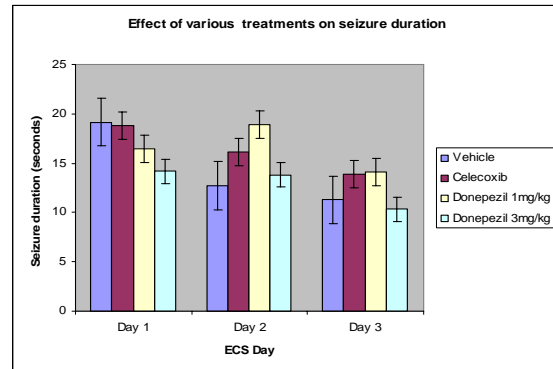
Final transfer latency in Seconds		
TREATMENT	TRUE	SHAM
Vehicle	39.66 ± 6.8	20.78 ±3.87*
Celecoxib	18.52 ±3.11#	20.97 ±4.94
Donepezil 1mg/kg	22.44 ±4.98	21.95 ±6.45
Donepezil 3mg/kg	19.77 ±2.74\$	19.19 ±2.23

Values are expressed as Mean±SEM. N=8. ANOVA, \*P<0.05 true vs sham, # P< 0.05 celecoxib true ECS vs vehicle true ECS, \$P< 0.05 Donepezil 3mg/kg true ECS vs vehicle true ECS



**Fig. 1: Effect of Galantamine on final transfer latency on ECS induced amnesia**

Values are expressed as mean ± SEM, n=6, one way ANOVA, \*p < 0.05 and \*\*\*p < 0.01 Vs control. Dunnett post hoc analysis



**Fig. 2: Effect of seizure duration on ECS induced amnesia**

Values are expressed as Mean±SEM. n=8. ANOVA. Main effect for time, Pillai's trace = 0.593, F = 21.11, df = 2, P < 0.0001. Drug x time interaction, Pillai's trace = 0.445, F = 2.86, df = 6, P = 0.016. Main effect for drug, Pillai's trace = 0.445, F = 1.32, df = 3, P = 0.29

**Table 2: Effect of Donepezil and Galantamine on Scopolamine induced amnesia**

Treatment	Baseline Transfer Latency in Seconds	Final Transfer Latency in Seconds
Vehicle	18.12±2.9	18.68±1.7
Scopolamine 0.5mg/kg	18.7±2.3	43.98±3.9
Donepezil 1mg/kg	17.95±1.7	12.09±1.0*
Donepezil 3mg/kg	19.30±2.6	6.57±0.9**
Galantamine 1mg/kg	19.45± 3.5	13.76±1.44*
Galantamine 4mg/kg	24.23± 1.4	9.6±0.96**

Values are expressed as Mean± S.E.M, n=8; ANOVA followed by Tukey's multiple comparison test, \* P≤0.01, \*\*P≤ 0.5 Vs Scopolamine

**Table 3: Effect of Donepezil and Galantamine on Locomotor Activity.**

Treatment	Locomotor Activity in Seconds
Vehicle	191.87±2.496
Scopolamine 0.5mg/kg,	194.37±4.009
Galantamine 1mg/kg+ Scopolamine 0.5mg/kg	188.12±3.7344
Galantamine 4mg/kg+ Scopolamine 0.5mg/kg	190.37 ± 2.803
Donepezil 1mg/kg + Scopolamine 0.5 mg/kg	187.87±7.53
Donepezil 3mg/kg + Scopolamine 0.5 mg/kg	189.25±5.86.

## DISCUSSION

Alzheimer's disease is a progressive neurodegenerative disease, characterized by deficits in memory and cognitive function. A remarkable dysfunction of the cholinergic system was seen in several brain regions of patients suffering from Alzheimer's disease and was shown to be correlated with the severity of cognitive impairment<sup>11</sup>. Dementia is one of the characteristics of Alzheimer's disease, Donepezil and Galantamine are the established drugs to treat dementia associated with Alzheimer's disease.<sup>12</sup> We sought to explore whether, Donepezil and Galantamine which is used to treat dementia of Alzheimer's type can be used to treat the cognitive impairments associated with ECT.

ECT introduces a new deficit in consolidation or attention, so that new information learnt is rapidly forgotten. During and shortly following ECT, patients display Retrograde amnesia (RA), retrograde amnesia describes the loss of previously acquired memories.<sup>13</sup> During ECT, in the immediate postictal period, patients may manifest with transient neurological abnormalities and disturbances in higher cognitive functions, particularly learning and memory.<sup>14</sup> Learning and memory are, inter alia, dependent on normal cholinergic neurotransmission; the relationship may be dose-dependent.<sup>15</sup> Electroconvulsive shocks (ECS) down regulate muscarinic cholinergic receptors.<sup>2,3</sup> This effect, therefore, may at least in part explain the cognitive deficits associated with the treatment.

In the present study, Donepezil and Galantamine were studied for its efficacy on ECS induced Retrograde amnesia. Cyclooxygenase mechanisms are involved in glutamate-mediated learning and memory as well as in glutamatergic excitotoxicity; hence, COX-2 inhibition would attenuate retrograde amnesia with ECT.<sup>13</sup>

Elevated Plus Maze was originally designed to evaluate anti-anxiety agents. Currently, it is also used to measure the cognitive performance, mainly spatial long term memory in rats and mice.<sup>16</sup> The post-ECS data shows that the length of time for the rats to enter the closed arm were large in the ECS vehicle group, but were smaller in the celecoxib and Donepezil 3mg/kg and Galantamine 4mg/kg groups. This demonstrates ECS induced retrograde amnesia in the ECS-vehicle group, and suggests Donepezil and galantamine prevented the development of amnesia. This result was statistically significant, as is evident from the significant ECS and drug interactions in the analysis of the post-ECS data. Since the groups of rats were comparable in seizure indices, these variables are unlikely to have influenced the findings.

The post-ECT recovery of cognition was more rapid in Rats administered with Donepezil 3 mg/kg and Galantamine 4 mg/ by exerting their anticholinesterase activity.

The cholinergic neurotransmitter system is involved in the storage and retrieval of new learning, as well as playing an important role in other aspects of cognition (eg, information processing speed). The storage and retrieval of new learning is supported by a complex circuit that involves large numbers of cholinergic projections from the nucleus basalis of Meynert (NbM) to the hippocampus, amygdala, and throughout the cortex. It is well known that lesions to these mesial temporal and basal forebrain cholinergic memory circuits lead to deficits in learning and memory.<sup>17</sup>

Donepezil and Galantamine being AchEI, Cholinergic facilitation is evident by the reversal of scopolamine-induced amnesia. The time taken by rats to enter the enclosed arm (TL) on the 2nd day was shorter than that on the 1st day in case of Donepezil and galantamine treated groups. From this we can infer that Donepezil 3mg/kg, galantamine 4mg/kg reversed the block of muscarinic receptors brought about by scopolamine.

Locomotor activity is performed to confirm if the drugs used in our study influences the motor activity in the animals and to validate if the drug is not influencing the movement of the animals in the EPM

where it should exert its effect only on memory consolidation. In our studies, we compared the locomotor activity with the control and were found that, locomotor activity is not affected by any of the drug treatment.<sup>18</sup>

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