ASSESSMENT OF MEMORY IMPAIRMENT ACTIVITY OF CLONAZEPAM ON MICE

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Keywords:
Antiepileptic drug,
Nootropic drug,
Clonazepam,
Ocimum sanctum,
Acetyl cholinesterase

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ABSTRACT

Memory impairment is a common side effect found in epilepsy and the anti-epileptic drugs (AED’s) used for treatment can aggravate the same. Nootropic drugs can potentially reduce memory impairment when combined with AED’s. Hence, the present study was aimed to assess the memory impairment activity of clonazepam (CLZ) in presence and absence of Ocimum sanctum Linn hydro alcoholic leaf extract (OSHAE).

OSHAE was prepared and subjected for in-vitro antioxidant activity by various methods. CLZ administered for twenty nine days has shown significant memory impairment in mice which was evaluated by Morris Water Maze (MWM) task on Maximal Electro Shock (MES) induced epileptic mice. In MWM method, animals were observed for Escape Latency Time (ELT) and Time Spent in Target Quadrant (TSTQ). Finally an acetylcholine-esterase (AChE) level in mice brain homogenate was estimated. OSHAE exhibited potent free radical scavenging activity when evaluated by in-vitro methods. CLZ increased ELT, decreased TSTQ when compared to vehicle treated group, indicating memory impairment due to its chronic administration which was reversed when OSHAE was given along with CLZ. The anticonvulsant activity of CLZ was found to be potentiated due to the co-administration of OSHAE when compared to vehicle and CLZ treated animals. The AChE levels in CLZ were found to increase when compared to vehicle and phenytoin group which was reversed by co-administration of OSHAE.
INTRODUCTION

Epilepsy is a very common disorder, characterized by seizures, which takes various forms and result from episodic neuronal discharges. The form of the seizure depends on the part of the brain affected [I]. Epilepsy affects 0.5-1% of the population. Epilepsy is treated mainly with drugs, although brain surgery may be used for a very few suitable severe cases. Current AED’s are effective in controlling seizures in about 70% of case[II]. Like all drugs, AED have side effects some of which are dose-dependent, and become most probable as the dose increases. Among other side effects like drowsiness, irritability, nausea, rashes, clumsiness, changes in emotions, impaired memory is the most common complaint of patients suffering from epilepsy [III]. In order to correct the side effect taken into consideration, nootropic co-administration was essential keeping in account of retaining the anticonvulsant effect of the AED used for treatment[IV]. OSHAE was found to be a potent anticonvulsant nootropic based on previous findings [IV]. Various studies indicate that the increase in brain AChE levels increase the degree of memory impairment when assessed by tests of memory and information [V]. Based on the assembled literature this study was concerned to evaluate the memory impairment potential of CLZ when administered to mice and the protective effect of OSHAE on CLZ induced memory impairment upon co-administration.

MATERIALS AND METHODS

Animals: Albino mice of either sex (20-35 g) procured from the Central Animal House Facility of JSS Medical College, Mysore were used for the study. The animals were housed in polypropylene cages at 23–27°C with a natural light-dark cycle. The mice were fed on a standard mice pellet diet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions for a week period before the start of the experiment. Groups of 6 mice were used in all sets of experiments. All experiments were in accordance with approval of Institutional Animal Ethics Committee (IAEC) of JSSCP Mysore; project number is 138/2013.

Chemicals: Aluminium chloride, ascorbic acid, chloroform, clonazepam, methanol, phenytoin, sodium carbonate, sodium chloride etc.

Drugs and plant extract: Clonazepam from Torrent Pharmaceuticals Ltd, Gujarat, India was obtained as gift samples. The Hydro alcoholic extracts of leaves of Ocimum sanctum (OSHAE) were procured from Sri Nidhi Industries, Mysore, Karnataka as gift samples.

Preliminary phytochemical screening [VI-IX]: The preliminary phytochemical screening was carried out on OSHAE in order to find out the presence of phytochemical constituents.
Pharmacognostical evaluation\[XI]:
  i. Total phenolic content
  ii. Total flavonoid content

*In-vitro* antioxidant and free radical scavenging activity of OSHAE: The OSHAE was subjected to different *in-vitro* antioxidant methods as shown below.
  i. DPPH free radical scavenging assay\[XI]
  ii. Superoxide dismutase\[XII]
  iii. Hydroxyl radical scavenging assay\[XIII]

*In-vivo* studies:

**EXPERIMENTAL METHODS**

**Table 1. Memory impairment activity of CLZ by Morris Water Maze model on MES induced convulsions. (Treatment schedule)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal + MES</td>
<td>Sodium CMC (0.5%) as a vehicle was administered orally for 29 days and convulsions induced by MES method on 0(^{th}), 8(^{th}), 15(^{th}), 22(^{nd}) and 28(^{th}) day.</td>
<td>Antiepileptic activity by MES method was assessed on 7(^{th}), 14(^{th}), 21(^{st}) and 28(^{th}) day and memory impairment activity by MWM method was assessed on 8(^{th}), 15(^{th}), 22(^{nd}) and 29(^{th}) day. Finally AchE level was assessed in brain homogenate on 30(^{th}) day.</td>
</tr>
<tr>
<td>Phenytoin+MES</td>
<td>Phenytoin (25 mg kg(^{-1})) was administered orally as 0.5% Sodium CMC suspension for 29 days and convulsions induced by MES method.</td>
<td>-do-</td>
</tr>
<tr>
<td>CLZ+MES</td>
<td>CLZ(5mg kg(^{-1})) was administered orally as 0.5% Sodium CMC suspension for 29 days and convulsions induced by MES method.</td>
<td>-do-</td>
</tr>
<tr>
<td>CLZ+OSHAE+MES</td>
<td>CLZ(5mg kg(^{-1})) and OSHAE (200 mg kg(^{-1})) was administered orally as 0.5% Sodium CMC suspension for 29 days and convulsions induced by MES method.</td>
<td>-do-</td>
</tr>
<tr>
<td>OSHAE+MES</td>
<td>OSHAE (200mg kg(^{-1})) was administered orally as 0.5% Sodium CMC suspension for 29 days and convulsions induced by MES method.</td>
<td>-do-</td>
</tr>
<tr>
<td>CLZ(1/2)+OSHAE+MES</td>
<td>CLZ (1/2) dose (2.5mg kg(^{-1})) and OSHA(200mgkg-1) administered orally as 0.5% Sodium CMC suspension for 29 days and convulsions induced by MES method.</td>
<td>-do-</td>
</tr>
</tbody>
</table>
Memory impairment activity of CLZ by Morris water maze (MWM)

The MEZ induced epileptic model animals were used for the MWM task. On 8th, 15th, 22nd and 29th day of treatment, the animals were evaluated for the memory impairment caused by CLZ Then on 29th day, same animals were sacrificed and the AchE activity was estimated in brain homogenate.

**Procedure:**

The animals were divided into 6 groups of 6 animals each as shown in Table 1. The mice were released into the water and allowed 90 s to find the platform. If mouse does not find platform after 90s the animal were guided by putting finger on top of the platform and then allowed mouse to sit there for 10s. Then the animals were placed in the heated cage for 60s and started the next trial. Then the animals were returned to home cage after 4 trials. In general animals received three trials per day with five minutes inter-trial interval for seven days or until the performance was stable.

Time to find the hidden platform was considered as escape latency time (ELT). The platform in the water maze was kept at the same position throughout the test to assess the effect on spatial reference memory. Time spent in target quadrant (TSTQ) which is defined as the time spent in the quadrant that previously contained the hidden platform was also measured.

**Evaluation of Anticonvulsant activity**

**Procedure:** The animals were divided into 6 groups of 6 animals in each group as shown in Table 1. The animals were treated as per the schedule for 29 days. Anticonvulsant potential of CLZ in presence and absence of OSHAE was assessed on 7th, 14th, 21st and 28th day. Tonic and clonic convulsions were induced by giving MES (45 mA for 0.2 s) using an electroconvulsiometer (Inco electroconvulsiometer) via ear electrodes. Duration of (a) flexion, (b) extension, (c) clonus, (d) stupor and (e) recovery or death of animal was recorded.

**Estimation of brain acetyl cholinesterase activity**:

**Preparation of reagent:** Ellman reagent [which is known as 5,5'-dithiobis-(2-nitrobenzoic acid); DTNB] can be prepared by oxidizing 2-nitro-5-chlorobenzaldehyde to the carboxylic acid, introducing the thiol via sodium sulphide, and coupling the monomer by oxidization with iodine. [4]

<table>
<thead>
<tr>
<th>Table 2. Reagents for acetyl cholinesterase estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagents</strong></td>
</tr>
<tr>
<td>Phosphate buffer solution</td>
</tr>
<tr>
<td>Supernatant</td>
</tr>
<tr>
<td>DTNB</td>
</tr>
</tbody>
</table>
**Procedure:** The mice were euthanized; brains are removed quickly and placed in ice-cold saline. Frontal cortex was quickly dissected out from brain on a Petri dish chilled on crushed ice. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). 0.4ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in the absorbance per minute is thus determined.

The enzyme activity is calculated by following formula

\[ \Delta A \times \frac{V_t}{C \times b \times V_s} \]

Where

- **ΔA**: Change in absorbance
- **Vt**: Total volume (3.1)
- **C**: 13610*10^4
- **b**: Path length(1Cm)
- **V_s**: Sample volume (0.4ml)

The final reading of enzyme activity is expressed as µmoles/minute/mg protein

**RESULTS:**

**Preliminary phytochemical screening:** Preliminary phytochemical analysis of OSHAE revealed the presence of following phytochemicals:

- Alkaloids
- Flavonoids
- Tri-terpenoids
- Tannins
- Reducing sugar
- Saponins

**Pharmacognostical evaluation:**

The phenolic content in OSHAE was found to be 22.97±0.0005mg gallic acid equivalent/200mg extract and the flavonoid content in OSHAE was found to be 74.06±0.0058 mg quercetine / 200mg extract as shown in the Table 3.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic gm/mg gallic acid</th>
<th>Total flavonoid gm/mg quercetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSHAE</td>
<td>22.97±0.0005</td>
<td>74.06 ±0.0058</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, n=3

**In-vitro antioxidant and free radical scavenging activity of OSHAE:**

**DPPH radical scavenging assay:** Free radical scavenging activity of OSHAE by DPPH radical scavenging assay is shown in Table 4. OSHAE was found to be a potent scavenger of
DPPH radical with the IC_{50} of 172.9±8.32 μg/ml value of compared to ascorbic acid, used as standard which showed IC_{50} value of 4.70±0.25 μg/ml.

**Superoxide anion radical scavenging (SO) assay:** Free radical scavenging activity of OSHAE by Superoxide anion radical scavenging assay is shown in Table 4. OSHAE was found to be a potent scavenger of superoxide anion radical with the IC_{50} of 168.71±12.26μg/ml value of compared to ascorbic acid, used as standard which showed IC_{50} value of 5.59±0.04μg/ml.

**Hydroxyl radical scavenging assay:** Free radical scavenging activity of OSHAE by hydroxyl radical scavenging assay is shown in Table 4. OSHAE was found to be a potent scavenger of hydroxyl radical with the IC_{50} of 210±0.60 μg/ml value of compared to ascorbic acid, used as standard which showed IC_{50} value of 31.64±0.30 μg/ml.

<table>
<thead>
<tr>
<th>Methods</th>
<th>IC_{50} values (μg/ml concentration)</th>
<th>OSHAE</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH method</td>
<td>172.90±8.32</td>
<td>4.70±0.25</td>
<td></td>
</tr>
<tr>
<td>Super oxide anion method</td>
<td>168.71±12.26</td>
<td>5.59±0.04</td>
<td></td>
</tr>
<tr>
<td>Hydroxyl radical method</td>
<td>210.00±0.60</td>
<td>31.64±0.30</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, n=3

**In-vivo studies:**

Evaluation of memory impairment activity of Clonazepam by Morris water maze model (on MES induced convulsions):

The effect of OSHAE on CLZ induced memory impairment is represented in Table 5 and 6. Activity was done by Morris water maze using mice as experimental animal. Escape latency time (ELT), time spent in target quadrant (TSTQ) and whole brain acetyl cholinesterase activity were parameters used to assess the memory impairment potential of CLZ.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DAY 0</th>
<th>DAY 8</th>
<th>DAY 15</th>
<th>DAY 22</th>
<th>DAY 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13.67±0.71</td>
<td>12.17±0.74</td>
<td>11.17±0.60</td>
<td>9.66±0.66</td>
<td>8.83±0.40</td>
</tr>
<tr>
<td>PHT</td>
<td>15.33±0.55</td>
<td>18.00±0.51(^a)</td>
<td>42.00±2.36(^a)</td>
<td>56.00±1.00(^a)</td>
<td>71.50±2.14(^a)</td>
</tr>
<tr>
<td>CLZ</td>
<td>14.00±0.44</td>
<td>15.67±0.21(^a)</td>
<td>53.33±2.61(^b)</td>
<td>81.00±3.94(^a)</td>
<td>87.83±1.37(^a)</td>
</tr>
<tr>
<td>CLZ+OSHA E</td>
<td>15.33±0.21</td>
<td>14.98±0.25(^b)</td>
<td>13.67±0.71(^b,c)</td>
<td>12.00±0.57(^b,c)</td>
<td>9.23±0.77(^b,c)</td>
</tr>
<tr>
<td>OSHAE</td>
<td>13.00±0.51(^b)</td>
<td>15.17±0.47(^b)</td>
<td>14.00±0.51(^b,c)</td>
<td>11.50±0.56(^b,c)</td>
<td>8.83±0.74(^b,c)</td>
</tr>
<tr>
<td>CLZ(^1/2)+OSHA E</td>
<td>13.00±0.36(^b)</td>
<td>15.00±0.36(^b)</td>
<td>12.00±0.36(^b,c)</td>
<td>11.00±0.36(^b,c)</td>
<td>8.00±0.36(^b,c)</td>
</tr>
</tbody>
</table>

Values are Mean ±SEM, n=6, Statistical analysis - One way ANOVA
\(^a\) - Significant when compared with normal treated animals (P<0.05)
\(^b\) - Significant when compared with phenytoin treated animals (P<0.05)
\(^c\) - Significant when compared with clonazepam treated animals (P<0.05)
In this study it was observed that when CLZ (5 mg/kg) administered orally for 29 days on mice with MES induced convulsions, there was a significant increase in ELT (87.83±1.37 seconds) when compared to the normal group (8.83±0.40 seconds) on 29th day as shown in Table 5. Memory impairment of CLZ was found to be almost similar to PHT (an antiepileptic drug that has been proven to produce memory impairment in animal models)\(^{18}\). It was observed that administration of OSHAE at the dose 200 mg/kg along with CLZ with MES induced convulsions resulted in a significant decrease in ELT value (9.23±0.77 seconds) when compared to the CLZ, PHT as well as normal treated group on 29th day. It was observed that administration of CLZ half dose 2.5 mg/kg along with OSHAE with MES induced convulsions resulted in a significant decrease in ELT of 8.00±0.36 seconds when compared to the CLZ, PHT and normal treated group. The memory impairment induced by CLZ was found to be dose and duration dependent. Finally it has been proven that OSHAE has shown improvement in learning and memory in mice brain and reversed the CLZ induced memory impairment.

### Table 6. Effect of herbal nootropic- OSHAE on CLZ induced memory deficit in mice (time spent target quadrant in seconds)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DAY 0</th>
<th>DAY 8</th>
<th>DAY 15</th>
<th>DAY 22</th>
<th>DAY 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.33±0.33</td>
<td>15.33±0.33</td>
<td>17.17±0.47</td>
<td>18.50±0.34</td>
<td>19.00±0.25</td>
</tr>
<tr>
<td>PHT</td>
<td>15.17±0.30</td>
<td>17.83±0.65</td>
<td>21.67±0.66</td>
<td>23.00±0.36</td>
<td>23.56±0.36</td>
</tr>
<tr>
<td>CLZ</td>
<td>16.67±0.21</td>
<td>20.67±0.33</td>
<td>22.83±0.40</td>
<td>24.42±0.25</td>
<td>24.42±0.25</td>
</tr>
<tr>
<td>CLZ+OSHAE</td>
<td>16.67±0.21</td>
<td>20.67±0.33</td>
<td>22.83±0.40</td>
<td>24.42±0.25</td>
<td>24.42±0.25</td>
</tr>
<tr>
<td>CLZ½+OSHAE</td>
<td>15.83±0.30</td>
<td>17.83±0.65</td>
<td>21.83±0.54</td>
<td>23.56±0.36</td>
<td>23.56±0.36</td>
</tr>
</tbody>
</table>

Values are Mean ±SEM, n=6, Statistical analysis- One way ANOVA

a - Significant when compared with normal treated animals (P<0.05)
b - Significant when compared with phenytoin treated animals (P<0.05)
c- Significant when compared with clonazepam treated animals (P<0.05)

The time spent in target quadrant (TSTQ) is another parameter recorded for evaluating memory deficit property of CLZ and its improving activity due to co-administration of OSHAE. It was observed that chronic administration of CLZ in mice for 29 days with MES induced convulsions resulted in significant decrease in TSTQ to 9.83±0.3 seconds when compared to the normal group of 19.00±0.25 seconds on 29th day. Memory impairment of CLZ was found to be almost similar to PHT in this parameter (an antiepileptic drug that has been proven to produce memory impairment in animal mode). Co-administration of OSHAE extract at the dose of 200 mg/kg with CLZ on MES induced convulsions resulted in a significant increase in TSTQ of 23.00±0.36 seconds when compared to the CLZ treated group (Table 6) on 29th day. OSHAE alone group showed significant increase in TSTQ...
24.42±0.25 seconds when compared to normal and CLZ. This shows that, the OSHAE has potent memory improving activity on CLZ induced memory deficit. The memory impairment induced by CLZ was found to be dose and duration dependent. The results hence prove the ability of OSHAE in reducing the memory deficit produced by anticonvulsant drug CLZ.

**Anticonvulsant activity by MES method**

Table 7. Anticonvulsant activity of CLZ in presence and absence of OSHAE on mice by MES induced convulsions (Various phases of convulsion in seconds) on 29th day

<table>
<thead>
<tr>
<th>Phases</th>
<th>Normal</th>
<th>PHT</th>
<th>CLZ</th>
<th>CLZ + OSHAE</th>
<th>OSHAE</th>
<th>CLZ ½ + OSHAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>5.48±0.054</td>
<td>1.99±0.44a</td>
<td>3.028±0.15</td>
<td>1.54±0.19c</td>
<td>1.55±0.24c</td>
<td>1.95±0.20e</td>
</tr>
<tr>
<td>Extensor</td>
<td>12.13±0.13</td>
<td>0.00±0.00b</td>
<td>2.19±0.72</td>
<td>0.00±0.00b,c</td>
<td>1.28±0.14</td>
<td>1.49±0.26</td>
</tr>
<tr>
<td>Clonus</td>
<td>20.72±0.07</td>
<td>6.2±0.61a</td>
<td>8.93±0.23b</td>
<td>2.79±0.54b,c</td>
<td>4.54±0.30c</td>
<td>5.05±0.36c</td>
</tr>
<tr>
<td>Stupor</td>
<td>31.51±0.49</td>
<td>14.4±0.69a</td>
<td>13.9±1.68</td>
<td>8.39±0.51b,c</td>
<td>14.21±0.81b,c</td>
<td>13.5±0.78b,c</td>
</tr>
</tbody>
</table>

Recovery/Death Recovery Recovery Recovery Recovery Recovery Recovery

Values are Mean ±SEM, n=6, Statistical analysis- One way ANOVA

a - Significant when compared with normal treated animals (P<0.05)
b - Significant when compared with phenytoin treated animals (P<0.05)
c- Significant when compared with clonazepam treated animals (P<0.05)

The anticonvulsant effect of CLZ is presented in Table 7. Flexion, extension, clonus, stupor, death or recoveries were the parameters evaluated in MES induced convulsion. When CLZ administered orally at the dose of 5mg/kg for 29 days, it significantly produced protection in extensor phase 2.19±0.72 seconds and decreased the duration of stupor to 13.9±1.68 seconds when compared to normal group on 29th day. It was also observed that when OSHAE were administered along with CLZ resulted 100% protection in extension phase (0.0±0.00) and decreased duration of stupor to 8.39±0.51 seconds when compared to normal group thus indicating synergistic phenomenon. CLZ half dose with OSHAE also showed synergistic activity. OSHAE alone treated group showed good antiepileptic activity as compared to normal group. OSHAE abolished the extensor and stupor phase. CLZ retained its protection on MES induced convulsion even when co-administered with nootropic herb OSHAE.

**Estimation of brain acetyl cholinesterase (AChE) activity**

Table 8.Estimation of brain AChE levels in MES model

<table>
<thead>
<tr>
<th>Group</th>
<th>AChE levels in µmoles/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9.83±0.24</td>
</tr>
<tr>
<td>PHT</td>
<td>20.44±0.43a</td>
</tr>
<tr>
<td>CLZ</td>
<td>24.9±0.33b</td>
</tr>
<tr>
<td>CLZ+OSHAE</td>
<td>10.52±0.38e</td>
</tr>
<tr>
<td>OSHAE</td>
<td>8.73±0.36b,c</td>
</tr>
<tr>
<td>CLZ ½+OSHAE</td>
<td>9.74±0.32b,c</td>
</tr>
</tbody>
</table>

Values are Mean ±SEM, n=6, Statistical analysis- One way ANOVA

a - Significant when compared with Normal treated animals (P<0.05)
b - Significant when compared with Phenytoin treated animals (P<0.05)
c- Significant when compared with Clonazepam treated animals (P<0.05)
The brain acetylcholine level is responsible for memory and acetyl cholinesterase (AChE) is the enzyme responsible for metabolism of acetylcholine. In this study we have determined the level of AchE in the whole brain homogenate (Table 8). It was observed that chronic administration of CLZ for MES induced convulsions resulted in a significantly increased AChE value 24.9±0.33 µmoles/mg protein when compared to the PHT group. When the OSHAE was co-administered with CLZ on MES induced convulsions it has significantly decreased AchE value 10.52±0.38 µmoles/mg protein when compared to CLZ treated group. Similarly when the OSHAE at the dose of 200mg/kg was administered with MES induced convulsions it significantly decreased AChE value 8.73±0.36 µmoles/mg protein when compared to the CLZ treated group. Memory impairment of CLZ was found very much similar to PHT. CLZ ½ dose with OSHAE showed AChE levels very similar to OSHAE alone treated group. This study clearly shows OSHAE has potent nootropic activity on CLZ induced memory impairment.

DISCUSSION

Epilepsy refers to a disorder of brain characterized by the periodic and unpredictable occurrence of seizures\textsuperscript{[XVI]} . It is chronic disorder mainly occurring in children and elderly patients. AED’S are used in long term treatment of epilepsy. AED’S are also used in other conditions such as pain or psychosis. Patient with epilepsy can have impaired cognitive abilities. Many factors contribute to this impairment, including the adverse effects of AEDs \textsuperscript{[XVII]}. Clonazepam is a member of the drug class known as benzodiazepines. The main side effect of CLZ is thinking/memory impairment\textsuperscript{[XVIII]} . The exact mechanism of action of CLZ on how it causes memory impairment is unknown. The probable mechanism may be it alters the cholinergic system by reducing the acetylcholine levels in the brain like phenytoin\textsuperscript{[XXI]}.

Many herbs have both nootropic as well as anticonvulsant activity which include Withania somnifera, Ocimum sanctum Linn \textsuperscript{[XX]} which can be used for improvement of memory deficit caused by anticonvulsant drugs as well as potentiate the anticonvulsant activity of the CLZ. By preliminary phytochemical screening it was found that OSHAE contain alkaloids, triterpenoids, tannins, flavonoids, cardiac glycosides and trace amounts of carbohydrates, which may have been responsible for the observed antioxidant activity. The polyphenols are a diverse group of phenolic compounds (flavanols, flavonols, anthocyanin’s, phenolic acids, etc.) when react with folin reagent give blue colour chromogen in alkaline media. The phenolic content in OSHAE was 22.97mg gallic acid/ 200mg extract. Flavonoids
in plants occur as glycosides and hydrolysis of the glycosides to aglycone and formation of aluminum chelate complex of aglycone with AlCl₃ is determined spectrophotometrically. The flavonoid content OSHAE was 74.06 mg quercetine/ 200mg extract.

DPPH is usually used as a substrate to evaluate anti-oxidative activity of antioxidant. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non-radical form DPPH by the reaction. The extract was able to reduce the stable radical DPPH to yellow colored Diphenyl Picryl hydrazine. OSHAE has shown potent free radical scavenging activity by DPPH method and IC₅₀ was found to be 195μg/ml. Ascorbic acid used as a reference standard showed scavenging potential with an IC₅₀ value of 2.85μg/ml as depicted in (Table 4). The free radical scavenging activity of OSHAE was found to be dose dependent. Superoxide anion is a weak oxidant it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Numerous biological reactions generate superoxide anions which are highly toxic species. Scavenging activity of OSHAE has shown potent activity with IC₅₀ of 168.78μg/ml when compared to Ascorbic acid. The % scavenging was found dose dependent as shown in (Table 4). Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to the cell, the model used is ascorbic acid- iron- EDTA model of OH generating system. This is totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. In this study, OSHAE shows better dose dependent prevention towards generation of hydroxyl radicals with IC₅₀ of 210μg/ml, depicted in (Table 4).

In MWM result showed that CLZ 5mg/kg p.o when administered for 29 days along with MES induced convulsions, significantly increased the ELT by 22.3%, 79.0%, 88.0% and 89.9% while decreasing TSTQ by 16.4%, 53.7%, 73.3% and 93.2% on 8th , 15th , 22nd , and 29th day of treatment respectively when compared to normal treated group. The result clearly demonstrates that, the CLZ adversely affected cognitive impairment in the MWM task in mice (Table 5 and 6). These findings show that, the CLZ treatment in mice induces memory-impairing effects. Our study supports the available literature regarding the memory impairment activity of CLZ. When OSHAE was given along with CLZ significant reversal in ELT by 24.2%, 23.0%, 22.3% and 4.3% and increase TSTQ by 5.5%, 9.6%, 10.7% and 14.6% on 8th, 15th, 22nd and 29th day of treatment respectively when compared to the normal
The memory impairment produced by CLZ is compared with phenytoin treated group which is already been proven for its memory deficit activity which produced increase in the ELT by 18.49% and decrease in TSTQ by 10.13% on 29th day. When OSHAE was given alone it significantly decreased ELT by 7.14%, 73.74%, 85.8% and 89.9% while increasing TSTQ by 29.4%, 45.9%, 53.2% and 59.7% on 8th, 15th, 22nd and 29th day of treatment respectively when compared to the CLZ alone treated group. It was observed that CLZ ½ + OSHAE produced significant decrease in ELT by 4.2%, 77.4%, 86.4% and 90.8% while increasing TSTQ by 26.1%, 41.7%, 51.7% and 58.1% on 8th, 15th, 22nd and 29th day of treatment respectively when compared to the CLZ alone treated group.

AChE inhibitor’s clinical efficacy is thought to result from prolonging the half-life of acetylcholine through inhibition of AchE. The whole brain AchE activity was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithio-bis-nitrobenzoate ions. The rate of formation of thiocholine from acetylthiocholine in the presence of tissue cholinesterase was measured using a spectrophotometer. The study revealed that the acetyl cholinesterase (AChE) level in CLZ treated animal was high (Table 11). It was observed that administration of CLZ along with MES induced convulsions resulted in a significant increase in AChE value of 60.5% when compared to the normal group. When the OSHAE was co-administered with CLZ in MES induced animals, it has significantly decreased AChE value by 42.2% compared to CLZ treated alone. When OSHAE was administered alone, it produced a decrease in AChE value by 35.0% when compared to CLZ treated alone. Similarly when CLZ ½ dose was given along with OSHAE it produced a significant decrease of AChE value by 31.1% when compared to CLZ treated alone. Here it was observed that OSHAE alone produced good nootropic effect when compared to CLZ+OSHA and CLZ alone. But CLZ ½+OSHA was found to decrease AChE value more than that of CLZ+OSHA proving the hypothesis of reduction in memory impairment by reducing the dose of AED under treatment without compromising the anti-epileptic activity of the same. The nootropic effect of OSHAE may be attributed to its antioxidant property. The maximal electro-shock (MES) induced convulsions in animal represent grand mal type of epilepsy. In MES convulsions electroshock is applied through the ear electrodes. Through cochlear nerve stimulation, cortical excitation is produced. The MES-convulsions are divided into five phases as (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor and (e) recovery or death. Drug which reduces or totally abolishes the
extension phase is considered to be antiepileptic drug. Some recent studies report anticonvulsant effects of OSHAE against MES and PTZ induced seizures. CLZ when administered alone decreased extensor by 81.9% when compared to normal group. We found the potentiating effects in OSHAE (200mg/kg) against MES when given along with CLZ as it decreased extensor phase by 100% when compared to CLZ alone group (Table 7). OSHAE alone group showed good antiepileptic activity as compared to normal by decreasing extensor phase by 91.0%. While CLZ ½+OSHAE decreased extensor phase by 70.7%. This clearly demonstrates the protective effect as well as synergistic effect of OSHAE on CLZ for grand mal type of epilepsy on which CLZ alone show less significant antiepileptic activity.

**CONCLUSION**

Epilepsy is a most common disease mainly occurring in children and elderly patients. Cognitive disorders are common in patients, who are under the treatment of epilepsy. In the present study we found that, chronic administration of CLZ for 29 days significantly produced memory impairment in mice. CLZ which belongs to benzodiazepine class of antiepileptics, known to affect the cognitive function. A well reputed nootropic herb OS evaluated for memory enhancing property on memory deficit produced by chronic administration of CLZ. This was done by employing Morris Water Maze task on MES induced convulsion using mice as an experimental animal. The nootropic activity was evaluated by recording escape latency time (ELT), time spent target quadrant (TSTQ) in MWM model.

The anticonvulsant activity of CLZ when co-administered with OS was evaluated using MES induced convulsion. CLZ adversely affected cognitive function which was observed by MWM task, where it significantly increased ELT and decreased TSTQ and increased the brain AChE level when compared to normal animals. When OSHAE was given along with CLZ, significant reversal of CLZ induced memory deficit was observed. Both the acquisition and retention of memory had shown improvement without affecting its anticonvulsant activity. The results provide evidence for potential corrective effect of OSHAE in cognitive deficit noted as an adverse effect in CLZ. Thus a conclusion can be made based on the observation and results obtained that OSHAE can be used for alleviating the memory impairment caused by antiepileptic drug CLZ. Both acquisition and retention of memory showed improvement without affecting its anticonvulsant activity. The results provide evidence for potential corrective effect of
OSHAE in cognitive deficit associated with CLZ. However further research is required to investigate the usefulness of these nootropic in various animal models. However, clinical studies are required to explore the full potential of OSHAE in correcting CLZ induced cognitive deficits and finding a place in the current AED therapy. Our study gives a platform for the further extensive research on nootropic for the correction of AED induced cognitive impairment. The future directive is to obtain reduction in dose of an AED when combined with anticonvulsant nootropic thereby reducing the extent or degree of adverse effect produced by the AED used for treatment. Preparation of a new formulation which consists of a synthetic AED as well as herbal/ synthetic nootropic.

ACKNOWLEDGEMENT

The authors sincerely thank Dr. H.G. Shivakumar, Principal. JSS College of Pharmacy, Mysore, JSS University for providing necessary facilities required for the research work.

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