Evaluation of nootropic potential of *Ocimum sanctum* Linn. in mice

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Dementia is one of the age related mental problems and a characteristic symptom of various neurodegenerative disorders including Alzheimer’s disease. Certain drugs like diazepam, barbiturates and alcohol disrupt learning and memory in animals and man. However, a new class of drugs known as nootropic agents is now used in situations where there is organic disorder in learning abilities. The present work was undertaken to assess the potential of *O. sanctum* extract as a nootropic and anti-amnesic agent in mice. Aqueous extract of dried whole plant of *O. sanctum* ameliorated the amnesic effect of scopolamine (0.4 mg/kg), diazepam (1 mg/kg) and aging induced memory deficits in mice. Elevated plus maze and passive avoidance paradigm served as the exteroceptive behavioral models. *O. sanctum* extract decreased transfer latency and increased step down latency, when compared to control (piracetam treated), scopolamine and aged groups of mice significantly. *O. sanctum* preparations could of beneficial in the treatment of cognitive disorders such as dementia and Alzheimer’s disease.

**Keywords**: Diazepam, Memory, *Ocimum sanctum*, Piracetam, Scopolamine.

Normal ageing is known to deteriorate memory in human beings. Oxygen free radicals, the harmful by-products of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer’s disease in elderly. Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior, but the resulting side-effects associated with these agents have made their applicability limited. Indian system of medicine emphasizes use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders.

In Ayurveda, *Ocimum sanctum* Linn. (Lamiaceae) popularly known as the sacred tulsi (holy basil) and has been in clinical use for centuries. The plant is erect, herbaceous, much branched, annual, 30-75 cm tall, found throughout India ascending up to 1,800 m in the Himalayas. The leaves possess anthelmintic, expectorant, diaphoretic and stimulant effects. An infusion of the plant is given in arthritis, toothache, ringworm infection and piles. The decoction of the root is given in genito-urinary disorders and malaria. It is also found to possess chemopreventive, antistress, anticonvulsant, antiulcer, anti diabetic, analgesic, antioxidant properties, and anticancer, immuno-modulatory and antiinflammatory activities. The present study has undertaken to assess the potential of aqueous extract of *O. sanctum* Linn. as a memory improving agent employing exteroceptive and interoceptive behavioral models in mice.

**Materials and Methods**

Plant material—*O. sanctum* Linn. (Family-Lamiaceae) was collected locally and was identified and authenticated at Department of Pharmacognosy, M. S. Ramaiah College of Pharmacy, Bangalore. A voucher specimen (OT/HS-238) has been deposited in the department. Powder (1 kg) of *O. sanctum* was extracted by simple maceration process using deionised water (0.1% ethanol). The crude extract was filtered and concentrated by rotavapour flash evaporator. The density of the extract was found to be 1.234 g/ml. The yield of the extract from crude powder of *O. sanctum* was 13%. A suspension was prepared using tween 80. The suspension of *O. sanctum* (OS) was diluted in distilled water, whereas

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injections of diazepam (Calmpos®, Ranbaxy, India), scopolamine (Sigma Aldrich, USA) and piracetam (Nootropil®, UCB India Ltd., India) were diluted in normal saline. The volume of oral and ip administration was 1 ml/100 g of mice.

Animals—Swiss mice of either sex weighing around 18 g (younger ones, aged 10-12 weeks) and 25 g (older ones, aged 28 weeks) were used. Animals were procured from disease free animal house of CCS Haryana Agriculture University, Hisar (Haryana, India). They were acclimatized to the laboratory conditions for 5 days before behavioural studies. The animals had free access to food and water and were maintained under 12:12 hr light and dark cycles. All experiments were carried out during day time from 0900 to 1400 hrs. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Elevated plus maze—The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The procedure and endpoint applied in the present study for testing learning and memory have been described earlier.

Passive avoidance paradigm—Passive avoidance behavior based on negative reinforcement (exteroceptive behavioural model) was used to examine the long-term memory. Step down latency (SDL) was defined as the time taken in seconds by the mouse to step down from wooden platform to grid floor with all the four paws on the grid floor. Out of 80 mice tested 52 mice showing SDL in the range (2-15 sec) during the first test were used for the second session and the retention test. Step-down latency (SDL) was recorded.

Scopolamine induced amnesia (Interoceptive behavioural model) Amnesia was induced by administration of scopolamine hydrobromide (0.4 mg/kg, ip) on 8th day and the TL recorded. Retention was recorded after 24 hr. OS (50, 100 and 200 mg/kg, po) and piracetam (200 mg/kg) were administered for 8 successive days. After 60 min of administration of the last dose on 8th day, diazepam (1 mg/kg, ip) was administered. TL was noted after 45 min of administration of diazepam and after 24 hr.

Locomotor function—Locomotor activity of control and drug-treated animals was measured with the help of Photoactometer (INCO, Ambala, India).

Statistical analysis—All the results were expressed as mean ± SE. The data were analyzed using ANOVA and Student’s (Unpaired) t test. P < 0.05 was considered as statistically significant.

Results and Discussion

Effect on locomotor activity—In the present study, OS (50, 100 and 200 mg/kg) did not show any significant change in the locomotor function of animals (score 222.6±8, 218±2 and 211±15) as compared to control group (score 216.4±12) when tested using a photoactometer.

Effect on transfer latency (TL) using elevated plus maze—Aged mice showed higher transfer latency (TL) values on first day and on second day (after 24 hr) as compared to young mice, indicating impairment in learning and memory (i.e. ageing-induced amnesia). Piracetam (200 mg/kg, ip) pretreatment for 8 days decreased transfer latency on 8th day and after 24 hr, i.e. on 9th day as compared to distilled water treated group, indicating improvement in both learning and memory. Scopolamine (0.4 mg/kg) and diazepam (1 mg/kg) increased TL significantly (P < 0.05) in young mice on first and second day as compared to control, indicating impairment of memory (Table 1).

OS (50 mg/kg and 100 mg/kg, po) decreased the TL on 8th day and 9th day in young and aged mice (P < 0.05) when compared to control groups. Higher dose of OS (200 mg/kg, po) more significantly enhanced the learning and memory of aged animals rather than the young mice as reflected by marked decrease in TL on 8th day and 9th day when subjected to elevated plus maze tests (Table 1). The higher dose of OS pretreatment for 8 days successively protected young mice (P < 0.05) against scopolamine, diazepam and ageing induced amnesia.

Effect on SDL using passive avoidance apparatus—O. sanctum extract (200 mg/kg, po) profoundly increased SDL significantly as compared to control group on the second day indicating improvement in memory of young mice. Further, this dose of OS reversed scopolamine-induced amnesia as
well, like in the elevated plus maze model (Table 1). Scopolamine hydrobromide (0.4 mg/kg, ip) significantly decreased SDL on second day after training, indicating impairment of memory. OS (200 mg/kg, po) administered orally for 8 days significantly reversed amnesia induced by scopolamine and natural aging (Table 2).

Alzheimer’s disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis that impair daily living. The present study indicates that O. sanctum possesses nootropic activity in view of its facilitatory effect on retention of acquired learning. The shortening of transfer latency by piracetam and O. sanctum indicated improvement in memory, which is in accordance with the hypothesis of Itoh et al. Both piracetam and O. sanctum meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit.

Nootropics are a class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals. Further, O. sanctum also reversed the scopolamine-induced impairment in learning and memory, when assessed on elevated plus maze and passive avoidance paradigms. Therefore, it seems that O. sanctum improved learning and memory probably because of enhanced cholinergic transmission.

Anti-inflammatory drugs may be inhibiting both, the onset and the progression of Alzheimer’s disease. Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock induced retrograde amnesia and also against amyloid deposits in the brain. Anti-inflammatory action of O. sanctum may also be contributing to the observed memory-enhancing activity. Oxygen free radicals, the harmful by-products of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer’s disease in elderly. O. sanctum is reported to possess antioxidant and antistress properties as well. Thus, a combination of anti-inflammatory, antioxidant, antistress and neuroprotective role of O. sanctum could all be leading to the net memory enhancing effect. Hence, it may be concluded that O. sanctum may be useful as a

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### Table 1—Effect of O. sanctum (OS) on transfer latencies of young and aged mice on elevated plus maze

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Transfer latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Young)</td>
<td>Distilled water</td>
<td>21.63±0.3</td>
</tr>
<tr>
<td>Piracetam</td>
<td>200</td>
<td>18.04±3.04 a</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>17.80±9.86 a</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0.4</td>
<td>21.80±2.92</td>
</tr>
<tr>
<td>OS</td>
<td>50</td>
<td>17.22±1.76 a</td>
</tr>
<tr>
<td>OS +</td>
<td>100</td>
<td>12.34±4.15 a</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>15.3±3.21 b</td>
</tr>
<tr>
<td>OS +</td>
<td>100</td>
<td>17.54±1.81 c</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0.4</td>
<td>21.43±1.92</td>
</tr>
<tr>
<td>Piracetam +</td>
<td>200</td>
<td>4.31 c</td>
</tr>
<tr>
<td>Diazepam +</td>
<td>200</td>
<td>3.04</td>
</tr>
</tbody>
</table>

P values: <0.05 as compared to a control (young group), b Diazepam treated group, c Scopolamine treated group, and d Control (aged group).

### Table 2—Effect of O. sanctum on step-down-latency (SDL) using passive-avoidance apparatus

<table>
<thead>
<tr>
<th>Group (mice)</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>SDL after 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>Control (DW)</td>
<td>10</td>
<td>112.1±3.2 a</td>
</tr>
<tr>
<td>Young</td>
<td>OS</td>
<td>50</td>
<td>248.1±6.4 a</td>
</tr>
<tr>
<td>Young</td>
<td>Scopolamine</td>
<td>0.4</td>
<td>16.2±2.19 a</td>
</tr>
<tr>
<td>Aged</td>
<td>Control (DW)</td>
<td>10</td>
<td>42.46±6.31</td>
</tr>
<tr>
<td>Aged</td>
<td>OS</td>
<td>50</td>
<td>48.18±6.29 c</td>
</tr>
<tr>
<td>Aged</td>
<td>Scopolamine</td>
<td>0.4</td>
<td>523.6±23.21 a,b</td>
</tr>
</tbody>
</table>

P values: <0.05 as compared to a control (young group), b Scopolamine treated group, and c Control (aged group).
nootropic agent in the treatment of various cognitive disorders.

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References