Research Article

COMMIPHORA BERRYI AMELIORATING EFFECT ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENTS IN MICE

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ABSTRACT

In the present study, we studied the effects of the ethanol extract of Commiphora berryi bark on drug-induced learning and memory-impaired mice using Y-maze task and passive avoidance task. On pretreatment with the ethanol extract of Commiphora berryi at 100mg/Kg p.o significantly ameliorated scopolamine-induced cognitive impairments which were revealed by the reversal in the reduction of spontaneous alteration in the Y-maze task and by significant improvement in latency time in passive avoidance task. This confirms the presence of bioactive phytoconstituents in Commiphora berryi which could be useful to decline the cognitive deficits in memory-impaired mice.

Keywords: Commiphora berryi, Acute toxicity, Y-maze, Passive avoidance, Alzheimer’s disease.

INTRODUCTION

Learning means obtaining knowledge and memory is considered as the retention of the acquired knowledge, which can be recalled as and when needed1. Memory is the capability of an individual to record sensory stimuli, events, information etc., which can be stored over a short or a long period of time and recalled when needed2. Decreased learning abilities and impairment of cognitive functions are the common problems arise in the case of old age and stressful conditions. These circumstances may directly lead to amnesia and dementia like Alzheimer’s disease (AD)3.

Pathological studies have revealed that the cholinergic system plays a vital role in learning and memory. In addition, a loss of forebrain cholinergic neurons, reduction in acetylcholine level in the cerebral cortex and hippocampus in Alzheimer’s Disease. A defect in cholinergic transmission led to the development of acetylcholinesterase (AChE) inhibitors to treat dementia associated with Alzheimer’s Disease4.

Scopolamine is a muscarinic cholinergic receptor antagonist which induces memory impairment and serves as a pharmacological tool in producing amnesia in animal models. Clinical investigations reveal that cognitive impairment connected with scopolamine is similar to that in Alzheimer’s Disease5,6. Acetylcholinesterase inhibitors have shown consistent efficacy in recovering memory and reversal of scopolamine-induced amnesia in an animal model. Currently, efforts are being made to develop a new therapeutic agent free from side effects from herbs to treat cognitive impairments7.

Various medicinal herbs are widely used to develop memory and cognitive function and to treat neurodegenerative diseases in traditional medicine8. Pharmacological effects of anti-amnesic property in herbs have been previously reported9. This research work is mainly focused on identifying its anti-amnesic effect in Commiphora berryi(Burseraceae). Commiphora berryi is a thorny shrub, a moderate size tree widely distributed in the dry forest of Andhra Pradesh, Tamil Nadu and Karnataka10,11. Furthermore, this herb has been shown to have anti-inflammatory12, antimicrobial, antioxidant, hepatoprotective13, smooth muscle relaxing, anticandidal, antymycobacterial, antischistosomal, molluscicidal, anticancer and antilucre activities14. It was previously reported that the bark extracts of Commiphora berryi contain flavonoids, polyphenols, glycosides, steroids, tannins, diterpenoids, triterpenoids and carbohydrates15,16.

In this study, we have initiated this work to assess the benefits of ethanol extract of Commiphora berryi for its possible behavioral effects in mice against amnesia induced by scopolamine in two models, Y-maze task and passive avoidance task, a factor that may result in the development of Alzheimer’s disease.

MATERIALS AND METHODS

Chemicals

Scopolamine hydrobromide (scopolamine), Eserine and all other chemicals were purchased from Sigma-Aldrich, India. Scopolamine and Eserine were dissolved in 0.9% saline solution for animal administration.

Plant collection and extraction

The bark of Commiphora berryi belongs to the Burseraceae family were collected in the month of July from Mathur, Tiruchirappalli, Tamil Nadu and authenticated by Botanical survey of India, South region, Coimbatore (BSI/SRC/5/23/2014-15/Tech/1000). A Voucher specimen of Commiphora berryi (Arn) Engl. was maintained at Anna University, BIT campus, Tiruchirappalli. The bark was dried in shade and ground to a...
coarse powder. The plant materials were subjected to continuous hot extraction in 80% ethanol using soxhlet apparatus. The solvents were evaporated under reduced pressure and then lyophilized. Dried extracts were stored in -20°C until testing.

Experimental animals

Adult Male Swiss albino mice weighing between 25-30g were housed under standard environmental conditions (25±1°C, 55±5% humidity and 12h/12h light/dark cycle). The animals were allowed free access to tap water and standard laboratory mice food. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our Institutional Animal Ethics Committee under the Committee for the Purpose of Control and Supervision of Experiments on Animals (India) CPSCEA (Ref. No AUROT/IAEC/NOV2013-006 Dt.21.11.2013 & Ref. No AUROT/IAEC/DEC2014-011 Dt.22.12.2014)

Phytochemical screening

Preliminary phytochemical screenings were carried out on the ethanol extract of Commiphora berryi using standard procedures as mentioned in Trease & Evans and Harbone17,18,19.

Acute toxicity studies

The acute toxicity study was performed in compliance with OECD guideline 423, as per the stipulated guidelines three animals in each group were involved20,21. All the test animals were fasted overnight (~12 h) and weighed before and after dosing. Oral dosage of the ethanol extracts of Commiphora berryi was administered in relation to the body weight of the fasted mice. 5, 50, 300 and 2000 mg/kg concentration of the ethanol extracts of Commiphora berryi was administered using oral gavage. The animals were regularly and individually observed for mortality and clinical signs after dosing at 0, 1, 2, 4, 5, 6 hours and on day 1 with special attention being given during the first 4 h. Thereafter, the observation was continued daily for a total of 14 days22. On day 15 all the mice involved in the study were euthanized by an overdose of chloroform and the internal organs were examined for any signs of abnormality.

Y-maze task

The Y-maze test was performed in a three-arm maze separated at angles of 120°. The length, width and height of the Y-maze are 40cm, 3cm and 12cm respectively. The floor and walls of the Y-maze were built of dark opaque polyvinyl plastic as previously described by Kim et al 200623. Mice were initially placed in arm A and allowed freely to explore arm B and C. The sequence and number of arm entries were recorded manually for each and every mouse over a period of 8 min. The sequence of arms entries representing all the three arms i.e., ABC, CAB, or BCA but not BAB, was recorded as an alteration to evaluate the percentage of triad from which short-term memory can be estimated24. An hour before the test, mice were administered with ethanol extracts of Commiphora berryi (12.5, 25, 50 and 100 mg/kg, p.o.) or eserine (10 mg/kg, p.o.) as a positive control. Cognitive impairment was induced by administration of scopolamine (1 mg/kg, i.p.) 30 min after the oral administration of ethanol extract of Commiphora berryi, eserine, or 0.9% saline solution. Control animals were administered with 0.9% saline solution only. After each and every test arms were cleaned with water to remove odors and fecal residues between the tests. The percentage alteration score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 which is represented by the following equation. The number of arm entries was used as an indicator of locomotor activity.

% Alternation = [(Number of alternations)/(Total arm entries -2)] X 100

Passive avoidance task

Passive avoidance task training and testing were carried out in identical illuminated and non-illuminated compartments. The illuminated compartment was fixed with a 100W bulb and the floor of the non-illuminated compartment was replaced with stainless steel rods spaced 1cm apart. These two compartments were separated by a door (5x5 cm). In the case of acquisition trial, mice were initially allowed to remain in the illuminated compartment for 10s and the door was opened to explore the other compartment for a period of 5min. This was repeated for three trials at an interval of 30min. After the trial, when the animal had stepped into the dark compartment. Through stainless steel rods, an electric foot shock of 0.5mA/3s was delivered to produce an aversive response in mice. An hour before the acquisition trial, ethanol extracts of Commiphora berryi at various concentrations of (12.5, 25, 50 and 100 mg/kg, p.o.) or eserine (10 mg/kg, p.o.) as a positive control were administered. Cognitive impairment was induced by administration of half an hour after the administration of ethanol extracts of Commiphora berryi, eserine, or 0.9% saline solution scopolamine (1 mg/kg, i.p.) were administered to induce cognitive dysfunction. Normal saline of 0.9% solution was administered to control animals. After 24 hours of acquisition trial, retention trial was carried out in the same mice. The mice were allowed to stay in the light compartment and the door to the dark compartment was opened. The time taken by the mice to enter the dark compartment was recorded as latency time. In the case of normal mice, it remembered the shock received on a previous day when they entered the non-illuminated compartment and hence they showed a suppressed behavior of exploring the non-illuminated compartment in the retention trial. The decrease in entry latency and decreased time spent in bright compartment suggested poor memory retention. It was assumed that when the mouse did not enter the non-illuminated compartment within a period of 180s, it confirms that the mouse had remembered the training trial23,25.

Statistical Analysis

Results of the behavioral studies are expressed as a mean ± standard error of the mean. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Dunnet test to evaluate significant mean differences between treatment groups. Values of p < 0.05 were considered significant. All statistical analysis was carried out using Graph Pad prism software.

RESULTS

Phytochemical analysis

The phytochemical investigation of the ethanol extract of barks of Commiphora berryi indicated the presence of flavonoids, glycosides, reducing Sugars, starch, tannins and terpenoids.

Acute toxicity studies

The toxic effect of ethanol extracts of Commiphora berryi on the appearance and the general behavioral pattern of mice are shown in Table 1 and Table 2 respectively. There was no toxic...
symptoms or mortality was observed. All animals involved in the study lived up to 14 days after the administration of ethanol extracts of Commiphora berryi at the dose level of 5, 50, 300 and 2000 mg/kg body weight. The behavioral patterns of animals were observed first 4 h and followed by 24 h after the administration and the animals in both vehicles treated and extract-treated groups were normal and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss. Based on the observations the ethanol extracts of Commiphora berryi is classified as Category –5 (Globally Harmonized System of Classification and Labeling of chemicals) and the LD₅₀ value was found > 2000mg/kg.

**Passive avoidance task**

Passive avoidance task was performed for testing the effect of Commiphora berryi on scopolamine-induced memory impairment. As shown in Figure 1, the latency did not show huge differences among any of the groups during the acquisition trial. In the retention trial, the latency time of the scopolamine-treated group for entering the dark compartment was significantly shorter than the control group, indicating memory impairment. The latency of retention trial reduced by scopolamine treatment was ameliorated with the treatment of Commiphora berryi (10, 12.5, 25, 50 and 100mg/kg) in a dose-dependent manner. Commiphora berryi treatment significantly reversed the scopolamine-induced reduction of latency time.

**Y - maze task**

The effects of Commiphora berryi on short-term memory function using Y-maze task was performed. As shown in Figure 2, scopolamine (1mg/kg, i.p.) significantly decreased the percentage of spontaneous alternation. The scopolamine-induced reduction of spontaneous alternation was significantly restored by the treatment with Commiphora berryi (10, 12.5, 25, 50 and 100mg/kg) in a dose-dependent manner, suggesting the improved memory. The total number of arm entries between the groups was not different suggesting that locomotion activity was not affected by scopolamine, eserine, or Commiphora berryi treatment. The average spontaneous alternation at 100mg/kg was significantly higher than the lower doses of Commiphora berryi.

**DISCUSSION**

In the present study, we reveal for the first time that the treatment of Commiphora berryi ameliorates scopolamine-induced memory impairments in mice. This effect experimented in the passive avoidance task and Y-maze test in mice. Furthermore, we found that Commiphora berryi does not produce any adverse effects or mortality in mice, when received Commiphora berryi for acute toxicity studies via orally.

Preliminary phytochemical screening of ethanol bark extract of Commiphora berryi has confirmed the presence of carbohydrate, phytosteroids, proteins, phenolic compounds, flavonoids, glycosides, reducing sugars, starch, tannins and terpenoids. Diverse secondary metabolites including terpenoids, steroids, flavonoids, sugars, lignans, etc. have been previously discovered in the Commiphora genus. This revealed the bark of Commiphora berryi having rich sources of secondary metabolites.

Donepezil, rivastigmine and physostigmine are the approved acetylcholinesterase inhibitors to slow the development of Alzheimer’s disease symptoms. Although, these drugs which are accepted for Alzheimer’s disease have restrictions due to non-selectivity, cholinergic side effects: hepatotoxicity, nausea, diarrhea and poor bioavailability. This motivated our interest has to develop natural product based drugs with more efficacy and minimal side effects.

Commiphora berryi was administered at the dose level of 2000 mg/kg in Swiss Albino Mice. Mortality and gross examination were monitored in harmony with OECD guidelines. Commiphora berryi did not generate treatment-related signs of toxicity or death in any of the animals experienced throughout the observation period. Consequently, no observed adverse effect levels (NOAEL) were recognized for 2000 mg/kg Commiphora berryi in mice under the circumstances of this study.

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>No of mice expired per group/total number of mice in group</th>
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<tbody>
<tr>
<td></td>
<td>Commiphora berryi</td>
</tr>
<tr>
<td>5</td>
<td>0/3</td>
</tr>
<tr>
<td>50</td>
<td>0/3</td>
</tr>
<tr>
<td>300</td>
<td>0/3</td>
</tr>
<tr>
<td>2000</td>
<td>0/3</td>
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<table>
<thead>
<tr>
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<th>Test group</th>
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<tr>
<td></td>
<td>4 hours</td>
<td>24 hours</td>
</tr>
<tr>
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<td>Normal</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mucous membrane</td>
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</tr>
<tr>
<td>Behavioural patterns</td>
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<td>Normal</td>
</tr>
<tr>
<td>Salivation</td>
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<td>Normal</td>
</tr>
<tr>
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<tr>
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<td>Normal</td>
</tr>
<tr>
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<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Coma</td>
<td>Not Observed</td>
<td>Not Observed</td>
</tr>
<tr>
<td>Tremors</td>
<td>Not Observed</td>
<td>Not Observed</td>
</tr>
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</table>
Figure 1. Graphical representation of the effects of the ethanol extract of the bark of *Commiphora berryi* on scopolamine-induced memory impairment observed from the passive avoidance task.

Ethanol extract of *Commiphora berryi*, mg - milligram, kg – kilogram. Values are expressed mean ± SEM, n=6. One way ANOVA followed by Dunnett’s multiple comparison test was done. *denotes the values are compared with Eserine + scopolamine treated group and *is P<0.05, ** is P<0.01 and *** is p<0.001.

Figure 2. Graphical representation of the effects of the ethanol extract of the leaves of *Commiphora berryi* on scopolamine-induced memory impairment observed from the Y-maze task

Ethanol extract of *Commiphora berryi*, mg - milligram, kg – kilogram. Values are expressed mean ± SEM, n=6. One way ANOVA followed by Dunnett’s multiple comparison test was done. *denotes the values are compared with Eserine + scopolamine treated group and *is P<0.05, ** is P<0.01 and *** is p<0.001.

Y-maze test can be used to assess amelioration of short-term memory impairment in mice. The long-term memory formation in mice can be decided by retention latency time obtained from passive avoidance task. If the time needed for the mice to enter the dark compartment is augmented, it indicates that the mice have retained electric foot shock experienced a day before.

In the present study, we have evaluated the potential precautionary effect of *Commiphora berryi*, to improve the scopolamine-induced memory shortfalls in mice. The percentage of spontaneous alternation in Y-maze test was evidently amplified (Figure 2) on oral administration of the ethanol extract of *Commiphora berryi* bark. In passive avoidance task, there is no considerable change in latencies during acquisition trial, while the reduction in step-through latency was appreciably ameliorated during the retention trial in a dose-dependent behavior (Figure 1).

Scopolamine a competitive antagonist of muscarinic acetylcholine receptor used as a standard drug for inducing cognitive dysfunction in animal models. The physiological process of learning and memory are mainly controlled by the cholinergic system. A cholinergic agonist can enhance memory whereas cholinergic antagonist can cause memory dysfunction. In the etiology of geriatric Alzheimer's disease patients, the
cholinergic hypothesis is involved. Few reports on Commiphora genus is previously reported which have a close interaction with Alzheimer’s disease. Commiphora wightii had ameliorated the scopolamine and streptozotocin-induced memory deficits in mice11. Sesquiterpenes isolated from the resins of Commiphora myrrha were reported for its neuroprotective effects12. Taken together, Commiphora berryi might have the potential benefits to relieve various neurodegenerative diseases.

CONCLUSION

To our knowledge, this is the first systematic evidence for the ameliorative effect in Commiphora berryi in scopolamine induced memory dysfunction in mice. The ethanol extracts of the Commiphora berryi bark showed significant ameliorative properties in improving memory in cholinergic impaired mice. Furthermore, Commiphora berryi treatment has not created any adverse effects or mortality in treated animals throughout the acute toxicity studies. Commiphora berryi may be listed as a new therapeutic option in the treatment of Alzheimer’s disease which acts as an enhancer of cholinergic activity.

ACKNOWLEDGEMENTS

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