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RESEARCH ARTICLE

Investigation on learning and memory-enhancing activity of *Saraca asoca* flower (Roxb.) Wilde in experimental mice

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ABSTRACT

Background: *Saraca asoca* flower (Roxb.) Wilde has been properly used in India for the treatment of uterine tonic and diabetes, and combination of flower and bark was used to treat some neurological disorders in Ayurvedic medicine. Our previous study on it showed a significant antiepileptic, antianxiety, and antidepressant activity; therefore, the present study is aimed to evaluate the memory-enhancing activity of *S. asoca* flower in animal models. **Aims and Objectives:** The present study was undertaken to study the evaluation of memory enhancement activity of ethanolic extract of *S. asoca* flower (Roxb.) Wilde (ESAF) in animal models. **Material and methods:** Ethanolic extract of flower ESAF was prepared by incessant method using Soxhlet and cold evaporator apparatus with respected temperature. ESAF in the doses 50 and 100 mg/kg body weight was administrated to albino mice by orally 7 days followed by evaluation for memory-enhancing activity using standard protocols of elevated plus maze and Morris water maze. The results are expressed as mean ± standard error mean. Statistical analysis was performed by one-way analysis of variance test followed by *post-hoc* Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant (Graph Prism Pad Version 7.1). **Results:** ESAF 50 and 100 mg/kg and standard piracetam (100 mg/kg) administered orally for 7 days protected the animals against scopolamine-induced learning and memory impairment. In Morris water maze test, animals treated with ESAF 100 mg/kg and the piracetam showed a significant reduction in escape latency period. **Conclusion:** ESAF showed significant learning and memory enhancement potential in animal models. The present study also concluded that the flower is a rich source of phytoconstituents such as flavonoids, glycosides, and proteins, which may be attributed to its anti-amnesic effect.

KEY WORDS: *Saraca asoca* Flower; Elevated Plus Maze; Morris Water Maze; Scopolamine

INTRODUCTION

Alzheimer disease (AD) is the most common form (estimating for around 60% of all cases) dementia in the World, with people developing dementia in every 3 s. There were an estimated 46.8 million people around the world living with dementia in 2015, and this number is held to be close to 50 million people in 2017 and about 9.9 million novel cases of dementia are diagnosed every year worldwide, implying one new case every 3.2 s. Previously, 58% of people were living with dementia in low- and middle-income countries, but in 2050, this will increase to 68%. There is the fastest progress in the elderly population in places such as China, India, and their South Asian and Western Pacific neighbors.[1]

Alzheimer's disease is associated with the incidence of disorientated signs and symptoms in the hippocampal areas

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of the brain. The AD mainly affects elder people, and it is estimated that, by 2050, more than 115 million individuals will be living with dementia.[1,2] The chief known risk factor is increasing age, and the mainstream of people with Alzheimer's are 65 and older. However, Alzheimer's is not just a disease of old age and around early-onset forms of the disease are associated with the definite genomic defect. While the etiology is unknown, hereditary factors clearly play a role in 10–15% of cases.[3] So far, there are no available treatments to stop or reverse the progression of the disease, which worsens as it progresses and eventually leads to death. The presently used medications to treat the disease address only its symptoms and with limited efficacy.

Dementia is due to loss of neurons in the hippocampus and cortex, with subcortical degeneration of ascending cholinergic neurons and pyramidal cells in cerebral cortex.[4] The primary cases of AD show common emotional features such as loss of short-term memory, inability to develop new information, mood swings, difficulty in finding words, forgetting names, frustration, hostility, and irritability. In severe cases, patients become totally lost, and sense of time and place disappears. Patients become totally dependent on others and eventually require comprehensive care. Owing to the patient's total dependency on others, placement in a nursing home with full-time nursing care becomes necessary. Thus, AD presents a significant problem in patient managing as well. It is believed that therapeutic intervention that could postpone the onset or progression of AD would intensely reduce the number of cases in the next 50 years.[3]

An epidemiological survey revealed that dementia or loss of memory is a major problem in Indian population.[4] The rate of dementia increases significantly with increasing age of a person, and this aging process in mammals is associated with a slow weakening of sensory as well as motor performances in the brain. The decline in sensory and motor performance has been attributed to the oxidative damage to the cellular lipids, proteins, nucleic acids, and imbalance of various neurotransmitter levels due to oxidative stress. Therefore, various antioxidant supplements and flavonoids components might be beneficial for preserving brain functions and forestalling the age-related deficits.[5]

Currently, five medications are used to treat cognitive problems of AD: Four are acetylcholinesterase inhibitors and one N-methyl-D-aspartate receptor antagonist. No medication has been clearly shown to delay or stop the progression of the disease.[6] These five medications do not often properly meet the therapeutic effects of patients suffering from comorbid psychiatric conditions, and the drawbacks of such drugs include unwanted side effects, incredible benefits, and moderate costs. Hence, herbal plants can be good sources to find new remedies for these disorders.[7] In the search for an alternative, more specific, and perhaps cost-effective therapy, research has been initiated to evaluate natural sources, and the report summarizes information about the phytochemical, biological, and molecular activities and clinical applications of these various plants to provide sufficient baseline information that could be used in drug discovery campaigns and development processes, thus providing novel evidence for use in AD.[8]

Herbal medicine compromises several options to modify the progress and symptoms of AD. There has been a new trend in the preparation and marketing of drugs based on medicinal plants, and their scientific and commercial significance appears to be gathering momentum in health-relevant areas. These plant-derived products are carefully standardized, and their efficacy and safety for a specific application have been demonstrated.[5,9,10]

Saraca asoca of "Caesalpiniaceae" family is known as a small- to medium-sized handsome evergreen tree. In Hinduism, term "Ashoka" means "one of that relieves pain and grief of women" is considered as a sacred tree. It has been widely used by folk medicine as anticancer, antioxidant, antibacterial, anti-inflammatory, antifertility, anti-arthritic, cardioprotective, larvicidal, antimutagenic/genoprotective, and antidepressant and also has been extensively used in Ayurveda, Unani, and Homeopathy practices.[11] The ethanol extract of *S. asoca* flower (ESAF) leaves and bark has been demonstrated to possess antidepressant effect in rodents subjected to forced swim test and tail suspension test.[12]

Hence, the present investigation was carried out for phytochemical screening, followed by evaluation of memory enhancement activity of ethanolic ESFA flower (Roxb.) Wilde in animal models.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

The fresh plant flower was collected during April (2015–16) from West Bengal. It was taxonomically identified and was authenticated by Dr. Mruthunjaya, Department of Pharmacognosy, JSS Pharmacy College, Mysuru, and herbarium of the plant is preserved for future references (Specimen Voucher No. SA-10601/Pharma). The collected flowers were washed and shade dried at room temperature for 7 days. Dried flower was coarsely powdered, and fine powder was separated. The coarse powder of flower (800 g) was subjected to extraction with ethanol by Soxhlet apparatus, and extracts were concentrated to dryness by vacuum. The extract was then weighed to calculate the percentage of yield in terms of air-dried crude material. The resultant ESAF was kept in the refrigerator for further use. Before administration, the extract was freshly prepared with normal saline, and three doses (50 mg/kg, 100 mg/kg, and 250 mg/kg) were selected based on the results of previous studies.

Experimental animals: Female mice weighing between 22 and 30 g were randomly selected from the breeding stock of Central Animal Facility of JSS Medical College, Mysuru. They were housed in polypropylene cages under standard condition 25 ± 3°C, humidity 45–55%, and 12/12 h light/dark cycle. They were given free access to food and water *ad libitum*. The animals were acclimatized for 7 days before the study. The study was conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals. The experiment protocols were approved by the Institutional Animal Ethical Committee of JSS Medical College, Mysuru (JSSMC/PG/13B10601), and procedures in this study were performed in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (261/PO/ReBi/2000/CPSCEA). All efforts were made to minimize animal suffering and the number of animals used in the experiments.

Animal grouping: Healthy female Swiss albino mice were randomly selected and divided into six groups. Each group contained six animals. Group I served as vehicle control, Group II received Scopolamine (0.4mg/kg) injection, Groups III, and IV were administered with ESAF 50, 100 mg/kg/p.o., Group V was treated with standard drug (Piracetam 0.4 mg/kg/i.p.) for 7 days. Group VI received the ESAF extract and Standard Piracetam for 7 days, followed by Scopolamine (0.4mg/kg, on 7th day) injection before testing. The following tests were employed for the evaluation of memory activity, 1 hr. after the administration of the respective treatments and the animals were used only once for each test.. The following tests were employed for the evaluation of memory activity 1 h after the administration of the respective treatments, and the animals were used only once for each test.

Chemicals used were ethanol, scopolamine, piracetam, and normal saline (Sun Pharmaceuticals).

Phytochemical Analysis

The extract obtained from the powdered flower of *S. asoca* was subjected to phytochemical tests to determine the presence of active metabolites using standard procedure.[9]

Assessment of Memory Activity

Elevated plus maze: The elevated plus maze consisting of two open arms (16×5cm) and two enclosed arms (16 cm × cm 5 × 12 cm) was used. The maze was elevated to a height of 25 cm. Mice were placed individually at the end of an open arm facing away from central platform, and time took by them to move from there to either of the closed arm (transfer latency [TL]) was recorded. If the animal did not enter into the closed arms within 90 s, it was gently pushed into one of the two closed arms and the TL was assigned as 90 s. The mice were allowed to explore the maze for another 10 s and then returned to its home cage. Retention of this learned task was examined 24 h after the 1st day trial. TL after 24 h was expressed as "inflexion ratio (IR)" using the formula described by Jaiswal and Bhattacharya (1992): IR= $(L_1-L_0)/L_{O,}$ Lo is the TL after 24 h, and L_1 is the initial TL in seconds. Healthy female, weight 25-30g, were divided into six groups consisting of 6 animals. Group I: Saline (10ml/kg) was administered orally for 7 days after 90 min of administration on 7th day transfer latency was recorded. Retention of learned task was examined after 24 h. Group II: Scopolamine HCL (0.4mg/kg) was injected before training. TL was recorded after 50 min of injection. Retention was examined after 24 h. Group III, & IV: ESAF extract (50 & 100mg/kg) was administrated orally for 7 days. TL was noted after 90 min of administration on 7th day and again after 24h. Group V: Standard received Piracetam (100mg/kg). TL was recorded after 90 min of administration for 7 days and again after 24 h. and Group VI: treated Piracetam (100mg/kg) for 7 days on 7th day after 90 min of extract administration, Scopolamine HCL (0.4mg/kg) and) was given TL was recorded after 45 min of injection and after 24 h.

Morris water maze (Morris, 1984; Bejar *et al*., 1999; Frick *et al*., 1995; Gordon *et al*., 1995): A spatial test was performed by method of Morris with minor modification. The water maze is a circular pool (120 cm in diameter and 50 cm in height) with a featureless inner surface. The pool was filled to a depth of 35 cm with water containing 500 ml of milk (25°C). The pool was divided into four quadrants of equal area. A white platform (6 cm in diameter and 29 cm in height) was then placed in one of the pool quadrants.

The first experimental day was dedicated to swimming training for 60 s without the submerged platform. During the 5 subsequent days, the mice were given two daily trials with intertrial interval of 30 min in the presence of the platform, it was permitted to remain on it for 10 s, if the mouse did not locate the platform within 120 s, and it was placed on the platform for 10 s. The animal was taken to its home cage and was allowed to dry up under room temperature.

During each trial session, the time taken to find the hidden platform (latency) was recorded. 1 day after the last training trial session, mice were subjected to probe trial sessions in which the platform was removed from pool, allowing the mice to swim for 120 s, to search for it. A record was kept on swimming time in the pool quadrant where the platform had previously been placed. Memory impairment was induced in the mice with scopolamine (0.4 mg/kg I. P.) at 60 min after treatment of test samples. Control group received normal saline.

Statistical Analysis

Data obtained by elevated plus maze were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered as statistically significant. The data obtained

by Morris water maze were analyzed using two-way ANOVA. The results were computed using GRAPH PRISM PAD version 7.

RESULTS

Phytochemical Testing

Preliminary screening revealed the presence of tannins, proteins, glycosides, carbohydrates, saponins, and flavonoids in ethanolic extract of the flower of *S. asoca.*

Acute Toxicity Study

The acute toxicity was carried out in adult Swiss albino mice by fixed dose method of the Organization for Economic Cooperation and Development guideline No-423.[9] Ethanolic extract of roots of *S. asoca* was given orally up to the dose level of 2000 mg/kg.[7]

Behavioral Studies

Elevated plus maze (EPM) model: Analysis of EPM data revealed that TL (F [5.30 =154.1], $P < 0.0001$) of 1st day reflected learning behavior of animals, whereas the 2nd day examination of TL is shown a reflected retention of information or memory (F [5.30] = 133.3; $P < 0.0001$). Scopolamine-treated group (0.4 mg/kg) was injected before training impaired learning significantly as indicated by improved TL. Combination group (piracetam+ scopolamine) is significantly reduced and the TL is comparable to standard. ESAF (50 and 100mg/kg) ($P < 0.0101$ and $P < 0.0172$) and piracetam (100 mg/kg) ($P < 0.0001$) administered orally for 7 days protected the animals from scopolamine-induced impairment in learning and memory [Table 1].

Morris water maze: The improvement effect of ESAF on special learning and memory process was evaluated by Morris water maze test. The escape latency for finding the hidden platform is depicted in Figure 1. There is an increase in escape latency in scopolamine group when compared to control in both the trials ($P < 0.01$) of one group of amnesia-induced animals and both showed reduced time to escape on to escape platform. The group treated with ESAF 50 mg/kg ($P < 0.4270$) did not show any significance when compared to control. However, ESAF 100 mg/kg ($P < 0.0389$) and combination groups ($P < 0.0156$) showed the significance which is respectively shown in Figure 1 in both the trials.

Table 1: Effect of ESAF on TL of mice using elevated plus maze

Groups	Treatments	TL 1/7th day	TL after 24 h	IR
I	Normal saline (10 ml/kg)	18.7±1.01	18.83±0.70	0.0566±0.005
II	Scopolamine (0.4 mg/kg)	43.17±1.188	59.5±2.54	0.213±0.01
III	Piracetam 100 mg/kg	5.83±0.7****	4.5±0.4****	0.241±0.009
IV	ESAF 50 mg/kg	13±0.8*	17±2.7ns	0.145±0.012
V	ESAF 100 mg/kg	13.33±1.16*	10.5±1.1*	0.136±0.02
VI	Piracetam+scopolamine	7.5±0.3**	7.33±0.9**	0.45±0.08

TL: Transfer latency, ESAF: Extract of *Saraca asoca* flower, p<0.001

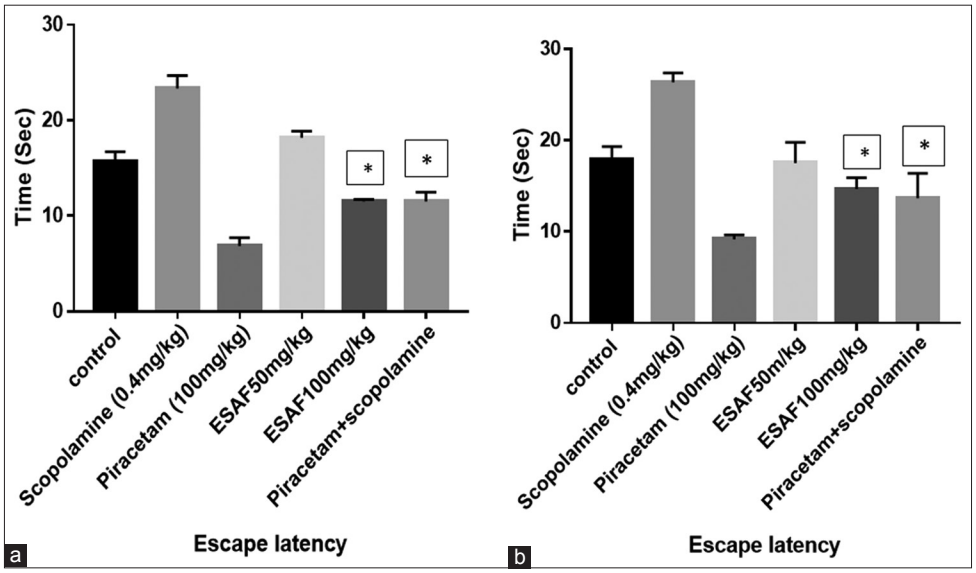

Figure 1: (a and b) Effect of ethanolic extract of *Saraca asoca* flower in elevated plus maze model at doses of 50 and 100 mg/kg and combination group in mice. Data represent mean ± standard error mean of escape latency of 6 animals in each groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to control group by one-way ANOVA followed by Dunnett's multiple comparison test

Data represent mean ± standard error mean of open arm spent time (in seconds) of six animals in each groups. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 compared to control group by one-way ANOVA followed by Dunnett's multiple comparison test.

DISCUSSION

The present study was aimed to demonstrate the pharmacological evaluation of ethanolic ESFA flower (Roxb.) against amnesic activity. To assess the efficacy, scopolamine was used to induce memory impairment in mice and impairment was evaluated using elevated plus maze and Morris water maze test.

Elevated plus maze was used to measure the anxiety state in animals, though TL was evidently concluded if animals had prior experience in open and closed arm, and this condensed TL has been shown to be associated with memory processes. Modern studies on numerous nootropics and amnesic agents on elevated plus maze have proven this model as an extensively accepted paradigm for training, learning, and memory process in rodents. In the elevated plus maze, learning can be considered as TL on the 1st day trials, and the retention/alliance (memory) is indicated by TL examined 24 h later. In our study, administration of piracetam and ESAF for 7 days protected animals from learning and memory impairment produced by interceptive stimuli (scopolamine). The verdict suggested the possible neuroprotective role for ESAF, whereas increase in IR after 24 h indicated an improved retention of the learned task.

In addition, the ESAF was evaluated to demonstrate its cognitive enhancing effects on spatial, memory, and learning function of mice against scopolamine-induced amnesic defects using Morris water maze test. In this test, ESAF and piracetam treatment on the scopolamine-induced amnesic mice exhibited significant shorter escape latencies in daily first trial than the scopolamine-administered groups during a 4-consecutive day training periods [Figure 1], which suggest that extract improved the impaired reference memory (long-term memory) induced by scopolamine. Furthermore, the formation of working memory (short-term memory) was revealed by significant differences in escape latencies between first and second trial on day 1 in treatment groups [Figure 1]. The results demonstrate that ESAF improves spatial learning and memory function against scopolamine-induced amnesia.

As mentioned, the phytochemical tests of ESAF exhibited the presence of various phytoconstituents such as flavonoids, saponins, and tannins.[13] It is known that saponin compound has nootropic and also antioxidant activities.[14] This moderately explains the mechanism of action of the extract. Further studies are warranted to isolate the nootropic compound and elucidate the mechanism that underlies spatial learning and memory, age-associated changes in spatial steering, and the ability of nootropic agents to influence specific cognitive processes. The neurochemical basis of learning and memory persist poorly understood despite extensive experimental and clinical study. Although the role of the central cholinergic system is fairly well recognized, the role of other neurotransmitter system cannot be ignored.[15] Since scopolamine-induced amnesia was reversed by ESAF, it is possible that the beneficial effect on learning and memory was due to the facilitation of cholinergic transmission in the mouse brain.

CONCLUSION

Memory impairment is the initial and most significant symptoms of AD. AD is associated with a decline in cognitive abilities and is the most common cause of dementia in the elderly. Despite the severity and high prevalence of this disease, the allopathic system is yet to provide a satisfactory antidote. The central cholinergic pathway plays a major role in learning and memory. In the present study, ESAF extract (100 mg/kg) administrated orally improved learning and memory in scopolamine-induced amnesia mice as assessed by behavioral models such as elevated plus maze and Morris water maze. Our previous studies showed significant antianxiety and antidepressant activities of ESAF due to its antioxidant property. Therefore, the present study concludes that the flower is a rich source of major flavonoids, which may be responsible for the anti-amnesia like CNS effects.

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