

**Research Article****Neuroprotective effect of *Sesbania sesban* on Scopolamine induced amnesia in wistar rats: Behavioral and biochemical study**

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**Abstract**

**Objective:** The present work was carried out to evaluate the effects of hydroalcoholic extract of *Sesbania sesban* (HAESS) on cognitive impaired rats. **Materials and methods:** In wistar rat's amnesia was induced by subcutaneous administration of scopolamine butyl bromide (1mg/kg). Nootropic activity evaluated in terms of Spatial memory using T maze spontaneous alternation task. **Results and conclusion:** HAESS (400mg/kg) have shown significant level improvement in scopolamine-induced deficit with respect to recognition in spatial memory. The observed results suggest that hydroalcoholic extract of aerial parts of *Sesbania sesban* improves cognitive performance with respect to spatial memory processes.

**Keywords:** Acetylcholinesterase, antioxidant, amnesia, T-maze, Scopolamine

**Introduction**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with loss of memory as one of the earliest symptoms. The pathological causes of this disease are numerous senile plaques composed of beta-amyloid (Ab) peptide, aberrant oxidative and inflammatory processes, neurotransmitter disturbances and cell loss in the affected brain regions, particularly in the areas that are important for learning and memory including the hippocampus and the prefrontal cortex (Villemagne and Burnham, 2013). Impairment of cholinergic neuronal system is consistently associated with memory loss and severity of Alzheimer's disease. It has been shown that selective and excessive loss of cholinergic neurons, deprived acetylcholine (ACh) levels, reduced numbers of cholinergic receptors in the brain cause the blockade of central ACh muscarinic receptors leads to disruption of learning and memory function in rodents, nonhuman primates and humans (Ishola et al., 2013). Scopolamine, A muscarinic receptor antagonist, interferes with a cholinergic neuronal system which ultimately affects the process of memory and learning functioning. Increased availability of ACh released

into the neuronal synaptic cleft has been used as a means of enhancing cholinergic function in an AD. This prolongation may be achieved by preventing or decreasing ACh hydrolysis by acetylcholinesterase inhibition (AChE). AChE inhibitors, including Piracetam, Tacrine, Memantine, and Galantamine, are well-accepted pharmacological therapies for an AD, But, these drugs have some limitations, such as short half-lives and severe side effects (e.g. Bradycardia, convulsions hepatotoxicity are most frequent and important side effect of these medications). Recent studies evaluated that AD is associated with inflammatory processes. Reactive oxygen species (ROS) are capable to damage cellular components and acts as a secondary messenger in process of inflammation. Use antioxidants may be useful in the treatment of Alzheimer's disease (AD).

*Sesbania sesban* (L.) Merr. commonly known as Jayanti, Jait, Shewari belongs to the family Fabaceae. It is a short-lived shrub or small tree up to 8 m tall, commonly grown as a shade plant for young seedlings grown during the hot season and as a windbreak for sugarcane. The plant belongs to the genus *Sesbania* and is common throughout Africa and in Asian countries (Gomase et al., 2012).

In present investigation our aim was to assess the Neuroprotective activity of HAESS in Scopolamine induced Amnesia in Rats Using T-maze alternation score task and Biochemical Oxidative Parameters (Andriambelosen et al., 2014).

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## Materials and Methods

### Chemicals

Scopolamine, Sodium Hydroxide, Acetylcholine Iodide, Trichloroacetic acid, 5,5-dithiobis (2-nitro-benzoic acid) (DTNB), Griess reagent, Thiobabutaric acid (TBA).

### Collection and identification of plant materials

Dried aerial parts of *Sesbania sesban* were obtained from Kedgaon, Maharashtra state, India. Plant was identified and Authenticated by Botanical Survey of India, Pune, a voucher specimen of the plant (Reference number BSI/WRC/100-2/Tech./2017/17) was deposited at the herbarium of BSI, Pune.

### Extraction of plant materials

The Powdered drug of *S. sesban* (50 gm) was extracted with 90% ethanol using solvent in soxhlet apparatus at 60°C under vacuum. The hydroalcoholic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rotary evaporator. The extract was dried in order to produce a dark brown solid extract.

### Animals

Wistar rats of either sex weighing 200–250g were used as the animal model in the experiment. The animals were provided by the central animal house facility of AISSMS College of Pharmacy, Pune, India. Animals were housed in groups of six in well-ventilated polypropylene cages with husk beds at an ambient temperature of  $25 \pm 2^\circ\text{C}$  and 60–65% relative humidity with 12 h light and dark cycle. They had free access to pellet chow and water ad libitum. The experimentations on animals were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No: CPCSEA/IAEC/PC-06/01-2K17) under the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. All the experiments were carried out between 9:00-14:00h.

### Preliminary acute oral toxicity

Healthy adult four female Wistar rats (200-250g) were subjected to acute toxicity studies as per guidelines (AOT 420) suggested by the Organization for Economic Co-operation and Development. The rats were observed continuously for 2h for behavioral and autonomic profiles and for any sign of toxicity or mortality for fourteen days.

### Experimental Design

Animals were randomly divided into 6 groups containing 6 animals each. Group 1 was taken as Normal control (neither Scopolamine nor HAESS), Group 2 were as Induction control where only Scopolamine 1 mg/kg/ s.c. injection was administered, Group 3 as Standard Treated group where Piracetam in dose of 200 mg/kg administered Orally. Groups

4,5,6 were taken as test Groups in which HAESS were administered in doses of 100,200 and 400 mg/kg p.o., respectively.

Groups 2,3,4,5 and 6 were treated orally for 14 days before injection of scopolamine (1mg/kg) subcutaneously on last day of treatment of standard and extract group. All the animals are subjected to behavioral evaluation 30 minutes after scopolamine injection followed by biochemical parameters.

### Experimental Protocol for Spatial Memory Assessment using T-maze task

The experiment protocol consisted of one single session, which started with one 'forced choice' trial, followed by 14 'free-choice' trials.

In the first trial, the 'forced-choice' trial, either the left or right goal arm was blocked by lowering the guillotine door. After the rat had been released from the start box, it would negotiate the maze, eventually enter the open goal arm, and return to the start position. There, the animal was confined for 5 seconds by lowering the guillotine door of the start box. During 14 'free-choice' trials, the rat could choose freely between the left and right goal arm. After opening the guillotine door of the start box, the animal was free to choose between both goal arms (all guillotine doors open). As soon as the rat entered one goal arm, the other goal arm was closed. The percentage of alterations over the 14 free choice trials was determined for each rat;

Percent spontaneous alterations =

(No. of spontaneous alterations / Total number of free-choice trials) x 100 % (Bartolini et al., 1992)

### Estimation of Biochemical Parameters

AChE and biochemical parameters of oxidative stress, MDA, glutathione (GSH) and nitrite, were measured in the brain of animals after the completion of behavioral studies.

### Brain Tissue Preparation

The rats were decapitated under ether anesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on ice. A 10% (w/v) homogenate of brain samples (0.03M ice cold sodium phosphate buffer, pH 7.4) was prepared by using a Tissue Homogeniser (Biolab) at a speed of 9500 rpm (Ishola et al., 2017).

### Acetylcholinesterase Assay in Brain

Reaction mixture containing brain homogenate (0.4 ml), 2.6 ml of ice cold phosphate buffer (0.1M, pH 8.0), and 100µl DTNB was mixed by bubbling air and placing it in a spectrophotometer. Once the reaction content was stable,

absorbance was noted at 412 nm for the basal reading followed by addition of 5.2µl of Acetylcholine Chloride to this cuvette. Any change in absorbance was recorded from zero time followed by 10 min at 25°C (Ellman et al., 1961).

#### Measurement of MDA

The 200µl of supernatant was added and briefly mixed with 1 ml of 50% trichloroacetic acid in 0.1M HCl and 1 ml of 26 Mm thiobarbituric acid. After mixing on a vortex, samples were maintained at 95 °C for 20 minutes, after which samples were centrifuged at 960g for 10 minutes and supernatants were read at 540 nm. The results were expressed at nmol/mg protein (Ellman et al., 1959).

#### Measurement of GSH

The 0.5 ml of brain homogenate was mixed with 0.1 ml of 10% trichloroacetic acid and centrifuged at 2000g for 10 minutes at 4°C. The supernatant was used for GSH estimation. To 300µl of processed tissue sample, 0.5 ml of phosphate buffer (0.1 M, pH 8.4), 0.2 ml of DTNB was added and the mixture was shaken vigorously on a vortex mixer. The absorbance was read at 412 nm within 15 minutes. The results were expressed at U/mg protein (Ellman et al., 1959).

#### Nitrite estimation

The 100µl of Griess reagent was mixed with 100 µl of the supernatant and vortexed. The absorbance was measured at 542 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite (0.01 – 0.1 mg/ml) (Ellman et al., 1959).

#### Statistical Analysis

The values were expressed as means ± SEM. Spontaneous alterations were assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

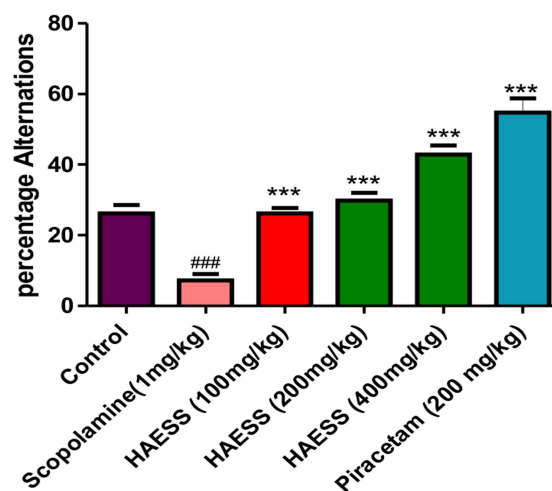
### Results

#### Preliminary Acute Oral Toxicity

No adverse effect or mortality was observed in wistar rats up to 2000 mg/kg, p.o of *Sesbania sesban* during the 24 hours and 14 days' observation periods.

#### Effect of hydroalcoholic extract of *S. sesban* on Scopolamine induced amnesia using T maze spontaneous alternation task

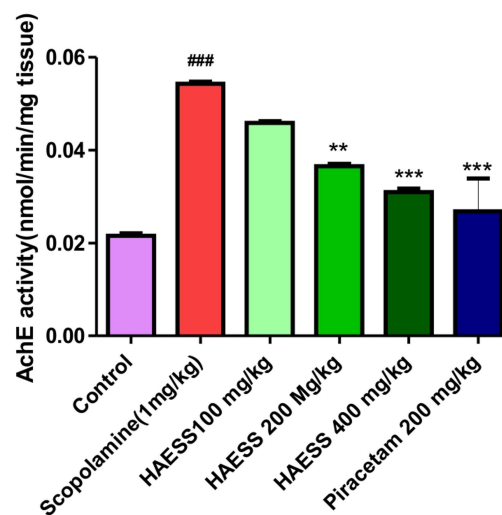
The effects of HAESS on short-term or working memory were investigated in the spontaneous alternation behavior in the T-maze in rats. Administration of scopolamine (1 mg/kg, s.c.) induced significant deficit in spontaneous alternation behavior when compared with vehicle-treated control. However, pretreatment of rats with HAESS reversed the decrease in alternation behavior induced by scopolamine. The highest dose of HAESS administered produced similar effects as the standard Piracetam group.



**Figure 1.** Effect of HAESS on spontaneous alternations behavior in the T-maze in rats. Values are expressed as Mean ± SEM ( $n = 6$ ), ###P < 0.0001 versus, control; \*\*\*P < 0.0001, versus scopolamine group (one-way ANOVA followed by Tukey's multiple comparison test).

#### Estimation of Brain Acetylcholinesterase (AChE) level

In this model, administration of scopolamine (1 mg/kg, s.c.) indicates high level in brain AChE level as compared to the control group. Administration of HAESS (100, 200 and 400 mg/kg) produced a significant decrease in AChE level when compared to the scopolamine group. Furthermore, Piracetam (200 mg/kg) showed a significant decrease in brain AChE level as compared with the Scopolamine group.

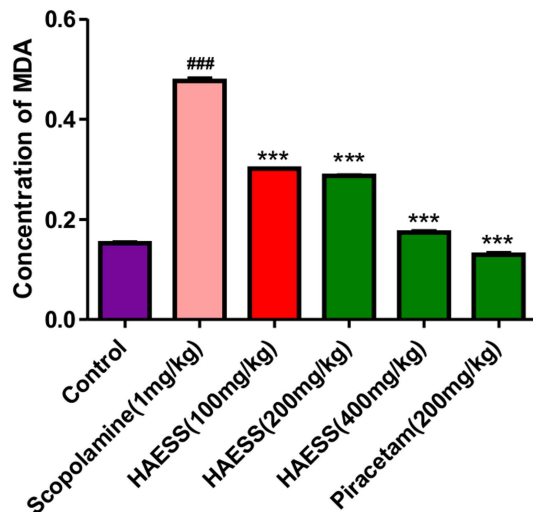


**Figure 2.** Effect of HAESS on brain AChE levels in Scopolamine-treated Rats. Values are expressed as Mean ± SEM ( $n = 6$ ), ###P < 0.0001, versus control; \*\*\*P < 0.0001, \*\*P < 0.001 versus scopolamine group (one-way ANOVA followed by Tukey's multiple comparison test).

#### Assay of Lipid peroxidation

Scopolamine administration (1 mg/kg, s.c.) significantly

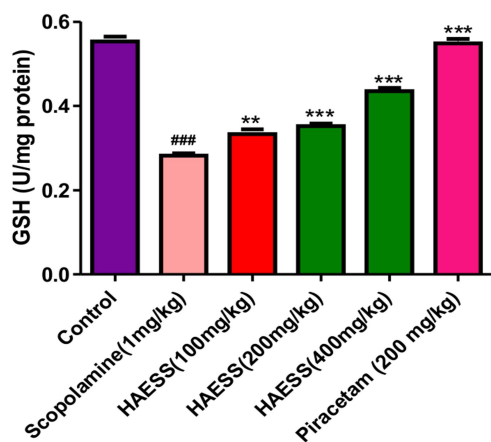
increased MDA (malondialdehyde) level in the rats in comparison with control group. Pretreatment of the rats with HAESS caused a significant decrease in MDA levels as compared to scopolamine group. The standard Piracetam also significantly decreased MDA levels in the rats as compared to scopolamine treated group.



**Figure 3.** Effect of HAESS on brain MDA levels in scopolamine-treated rats. Values are expressed as Mean  $\pm$  SEM ( $n = 6$ ), ###P < 0.0001, versus control; \*\*\*P < 0.0001, versus scopolamine group (one-way ANOVA followed by Tukey's multiple comparison test).

#### GSH (Glutathione) level estimation

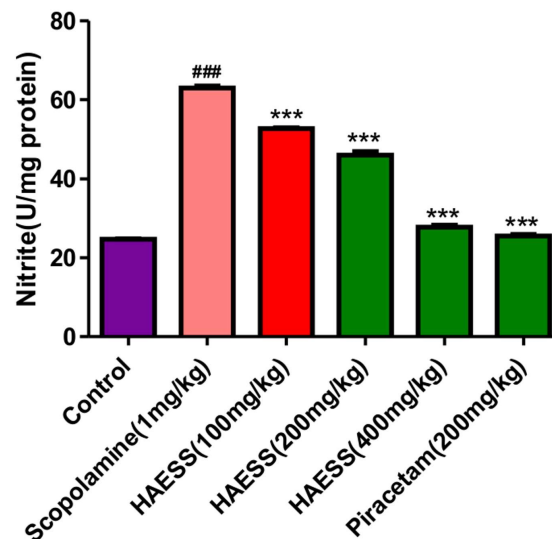
Scopolamine injection (1 mg/kg, *s.c.*) produced significant decrease in the GSH levels when compared with vehicle-treated group. Conversely, the pretreatment of rats with HAESS increased GSH level in the brains of treated rats as compared to scopolamine group. Scopolamine-induced deficit in GSH levels was also reversed by pretreatment of rats with Piracetam. However, pretreatment with 400 mg/kg of HAESS did yield significant results.



**Figure 4.** Effect of HAESS on brain GSH levels in scopolamine-treated rats. Values are expressed as Mean  $\pm$  SEM ( $n = 6$ ), ###P < 0.0001, versus control; \*\*\*P < 0.0001, \*\*P < 0.01, versus scopolamine group (one-way ANOVA followed by Tukey's multiple comparison test).

#### Nitrite estimation

The subcutaneous injection of Scopolamine (1 mg/kg, *s.c.*) significantly increased nitrite levels in the brain when compared to control group. However, pre-treatment of rats with HAESS decreased nitrite levels in the brain, when compared to scopolamine treated groups. Treatment with Piracetam showed significant decrease in nitrite levels in the brain.



**Figure 5.** Effect of HAESS on brain nitrite levels in scopolamine-treated rats. Values are expressed as Mean  $\pm$  SEM ( $n = 6$ ), ###P < 0.0001; versus control; \*\*\*P < 0.0001, versus scopolamine group (one-way ANOVA followed by Tukey's multiple comparison test).

#### Discussion

The cognitive-enhancing activity of Aerial parts of hydroalcoholic extract of *Sebania sesban* against scopolamine-induced memory impairments in rats was investigated using the T maze alternation task and biochemical assessments. In present investigation, scopolamine significantly reduced the alternation score in free choice trials, demonstrating that the central cholinergic neuronal system plays an important role in learning acquisition. Where groups treated with *S. sesban* improves alternation score along with Standard Piracetam (200 mg/kg) these results suggest that the anti-amnesic effects of *S. sesban* against scopolamine induced memory impairment may be related to mediation of the cholinergic nervous system. To elucidate the underlying mechanisms of memory enhancing effects of *S. sesban*, activity of AchE as cholinergic marker was assessed using brain homogenates. Neurochemical studies suggested that the cholinergic system plays an important role in learning and memory. According to the cholinergic hypothesis, memory impairments in patients with dementia are due to a selective

and irreversible deficiency in the cholinergic functions in the brain. Therefore, cholinesterase inhibitors may compensate for reduced ACh levels in brains with AD disease. In present study, *S. sesban* extract treatment significantly inhibited AchE activity. Thus, it could be explained that the anti-amnesic effect of *S. sesban* on scopolamine-induced impairment of learning and memory may be related to modification of cholinergic neuronal systems. This study further evaluated whether such impaired cognition by scopolamine is associated with altered oxidative stress indices. Scopolamine-treated rat had elevated MDA and Nitrite levels with reduced GSH activity. Increased MDA levels have been shown to be an important marker for in vivo lipid peroxidation. Oxidative stress results from a marked imbalance between free radical production and elimination by antioxidant systems. GSH is the principal intracellular non protein thiol and plays a major role in the maintenance of the intracellular redox state. The level of GSH diminishes with an increase in the generation of free radicals. In present study, MDA, Nitrite and GSH were estimated on the fifteenth day after the first injection of scopolamine. Scopolamine-treated rat showed a significant increase in MDA and decrease in the GSH level in the brain compared to control values, indicating elevated oxidative stress. However, Pretreatment with HAESS also affect the level of Oxidative Stress. The administration of HAESS produced significant reduction in MDA & Nitrite level, and restored the activities of GSH in rat brain. These observations suggest that hydroalcoholic extract of *S. sesban* produced significant antioxidant activity against scopolamine-induced oxidative stress.

### Conclusion

The findings in Present study showed that hydroalcoholic extract of *S. sesban* is potent neuroprotective agents which could be linked to the inhibition of AchE activity and Antioxidant activity in the brain of rat with scopolamine-induced amnesia. The Anti-AchE, antioxidant and anti-amnesic effects of hydroalcoholic extract of *S. sesban* on scopolamine-induced cognitive impairments in vivo suggest that they may be viable candidates for the treatment of neurodegenerative diseases, like Cognitive impairment.

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**Conflicts of interest:** None

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