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Securinega leucopyrus improves Memory and Learning in Alzheimer's Model: An Experimental Study in Rat

J. Gopi Chand*, S. Amreen Sultana, G. N. S. S. Chandra Sekhar, D. Eswar Tony, N. Rama Lakshmi, A. Narendra Babu, Rama Rao Nadendla

**Department of Pharmacology,
Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, India**

Abstract : Alzheimer's disease (AD) affects the central nervous system causing progressive degeneration of neurons, which affect cognitive function of the individual. So, the aim of this study was to identify the potential of aqueous and methanolic stem extracts of *Securinega leucopyrus* to be used as a therapeutic agent against Alzheimer's disease. The cognitive impairment was produced by the methods, ethanol- induced cognitive impairment &diazepam induced amnesia. The potentials of the extracts were determined by using Morris water maze (MWM) test. Both the extracts showed significant learning and memory enhancement activity.
Key words : Alzheimer's disease, *Securinega leucopyrus*, Ethanol, diazepam, Morris water maze.

Introduction:

Alzheimer's disease (AD), also referred to simply as Alzheimer's, is a chronicneurodegenerative disease, in which the death of brain cells causes memory loss and cognitive decline that usually starts slowly and worsens over time. It is the cause of 60–70% of cases of dementia.As the disease advances, symptoms can includeproblems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self care, and behavioural issues. As a person's condition declines, they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death. Although the speed of progression can vary, the typical life expectancy following diagnosis is three to nine years.Although current Alzheimer's treatments cannot stop Alzheimer's from progressing, they can temporarily slow the worsening of dementia symptoms and improve quality of life for those with Alzheimer's and their caregivers. Today, there is a worldwide effort under way to find better ways to treat the disease, delay its onset, and prevent it from developing[1].

Securinega leucopyrus (Family: Euphorbiaceae), popularly known as Bushweed and Indian Snowberry. It is a commonly found in India, Sri Lanka and Burma. It is a perennial shrub that grows up to 5 m in height.

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The genus Securinega is a native of Madagascar and the Mascarene Islands in Indian Ocean. There are about 45 species present in this genus. In the year 1789, Securinega was first described as a genus. Securinega was a genus in the family Phyllanthaceae, later it is changed to the family Euphorbiaceae.

It is used topically in paste form for healing of chronic and non-healing wounds[2]. The leaves of the plant contain germicidal properties. The decoction of leaves is used to dress the cancerous wounds and also used externally in the treatment of piles. The juice or paste of the leaves along with tobacco used to destroy worms in sores. It is used as popular veterinary medicine. The leaves are used to extract the extraneous materials from body tissues without surgery[3]. Leaves are boiled and taken twice a day for stomach aches. The roots are used in the treatment of testicular enlargement and in the cure of edema. The whole plant is used for the cure of cancer in the sole of the foot. It is also used in the treatment of abdominal lumps and liver hypertrophy and portal hypertension. The bark of stem is used for tooth ache[4].

Figure 1: Stems of *Securinega leucopyrus*

Materials and Methods

Collection & authentication of plant material:

The stems of the plant, *Securinega leucopyrus (Willd.)Muell.* was collected from the medicinal garden of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The plant material was identified and authenticated by Dr. M. Raghu Ram, Department of Botany, Acharya Nagarjuna University, Guntur.

Preparation of extract:

The stem of *Securinega leucopyrus* was shade dried and was powdered in a mechanical grinder. The collected powder was extracted with water & methanol by using Soxhlet apparatus. The extraction was carried out for 72 hrs. Excess solvent was removed by the solvent evaporation.

Experimental animals:

SD rats of either sex weighing between 200-300 gm were used. Animals were maintained under standard conditions in an animal house approved by Committee for the Control and Supervision of Experiments on Animals (CPCSEA Reg.No.: 1048/a/07/CPCSEA). The animals were housed in Poly propylene cages andmaintained at 25±2ºC under 12hlight / dark cycle and were fed with standard animal pellet feed (Hindustan lever limited) and water *ad libitum.* The animals were allowed to acclimatize to laboratory conditions for 48 h before starting the experiment.

Morris Water Maze (MWM):[5]

To access hippocampus dependent spatial learning and memory, all rats were trained in a standard Morris water maze task (Morris et al., 1982; Stackman et al., 2002). Maze consisted of large circular pool (75cm x 30cm) filled with water at a depth of 20cm, such that the platform is hidden under the water surface. The pool was divided into four quadrants. A circular platform was placed in one of the quadrants. The trial is performed for four consecutive days, at four trials per day, in which, the platform remains fixed and the starting

points differ as specified in Table 1. In the swimming trials, each individual rat was released gently into the water at a randomly chosen quadrant. The rats swim and learn how to find the hidden platform within 60 sec. After reaching the platform rat was allowed to stay on the platform for 15 s and was then taken back into the cage. The time taken by the rat to reach the platform was recorded as escape latency. If the rats could not escape to the platform within 60 s by themselves, then their escape latency was accepted as 60 sec. During the inter-trial intervals, animals were kept in a dry home cage. After the fourth trial of last day training, a probe trial was performed, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60 s trial.

Figure 2:Image of Morris Water Maze

Table 1: The sequence of trials during the study period of MWM test [6]

1st Day	2nd Day	3rd Day	4th Day
Q1	Q2	Q3	Q4
Q2	Q3	Q4	Q1
Q3	Q4	Q1	Q2
Q4	Q3	Q2	Q1

Ethanol- Induced Cognitive Impairment:

Ethanol is a neurotoxin that is able to alter behavioural and cognitive performance in experimental animals in addition to humans. It mainly impairs hippocampus-dependent learning and memory functions. The mechanism of ethanol-induced neurotoxicity is not well understood. Several studies show that free-radical mediated oxidative stress play an imperative role. The brain is extremely susceptible to oxidative stress due to high level of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O_2) in relatively small mass and in conjunction with low antioxidant activities. Furthermore, certain regions of the central nervous system (CNS), especially hippocampus and cerebellum, may be more sensitive to oxidative stress because of their low endogenous antioxidant, in relation to other brain regions. Study showed that acetaldehyde dehydrogenase is responsible for the generation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from oxidation of ethanol to acetate. It has been confirmed that ethanol induces the synthesis of CYP2E1 that lead to oxidative stress. It also increases the ratio of NADH/NAD, responsible for reduction of ferric ion (Fe^{3+})to ferrous ion (Fe^{2+}) which causes lipid peroxidation by generating hydroxyl radical.

Experimental design:

The learning and memory enhancing activity of the aqueous and methanolic stem extracts of *Securinega leucopyrus* was investigated using the ethanol- induced cognitive impairment [Ethanol (60%) is used to induce dementia like condition in the dose 2.25 mg/kg administered i.p for 15 days[7]]. The test animals were randomly chosen and divided into four groups each having five rats and the escape latency (EL) was noted on 1st, 7th, and 15th days.

Table 2: Grouping and treatment of the groups in ethanol induced cognitive impairment

S.No.	GROUPS	TREATMENT
I	**Negative control**	Ethanol (2.25 mg/kg was administered i.p for 15 days)
II	**Positive control**	0.9% Saline Solution
III	**Standard**	Donepezil hydrochloride (2.5 mg/kg[7] was administered orally for 15days) + Ethanol
IV	**Test-I**	Aqueous stem extract of *Securinega leucopyrus* [SLAE-100mg/kg was administered orally for 15days) + Ethanol
V	**Test-II**	Methanolic stem extract of *Securinega leucopyrus* [SLME-100mg/kg was administered orally for 15 days) + Ethanol

Diazepam Induced Amnesia:

Extracts and standard, donepezil hydrochloride were administered for 8 successive days and the escape latency was recorded. After 60 min of administration of the last dose on 7th day, Diazepam 1mg/kg i.p was administered. EL was noted after 45 min administration of diazepam and after 24 hrs[8-10].

Experimental design:

The learning and memory enhancing activity of the aqueous and methanolic stem extracts of *Securinega leucopyrus* was investigated using the Diazepam induced amnesia (Diazepam is used to induce amnesia like condition at a dose,1 mg/kg,i.p). The test animals were randomly chosen and divided into four groups each having five rats and the escape latency (EL) was noted on 7thday & after 24hrs of diazepam administration.

Table 3: Grouping and treatment of animals in Diazepam induced amnesia

S.No.	GROUPS	TREATMENT
I	**Negative control**	Diazepam (1mg/kg was administered i.p for 8 days)
II	**Positive control**	0.9% Saline Solution
III	**Standard**	Donepezil hydrochloride (2.5 mg/kg[7] was administered orally for 15days) + Ethanol
IV	**Test-I**	Aqueous stem extract of *Securinega leucopyrus* [SLAE-100mg/kg was administered orally for 15days) + Ethanol
V	**Test-II**	Methanolic stem extract of *Securinega leucopyrus* [SLME- 100mg/kg was administered orally for 15 days) + Ethanol

Statistical analysis:

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between

haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

Results and Discussion:

Effect of stem extracts of *Securinega leucopyrus* on behavioural Parameters:

Animals were treated with ethanol [2.25 mg/kg] for 15 days and with diazepam [1mg/kg]for 8 days in Ethanol induce cognitive impairment and Diazepam induced dementia respectively and showed a slight increase in escape latency in seconds. On 1st,7th& 15th day inEthanol induce cognitive impairment and 8th day & after 24 hrs i.e. 9th day in Diazepam induced dementia.

Table 4: Effect of stem extracts of *Securinega leucopyrus* on ethanol- induced cognitive impairment

S.No.	Group	Treatment	Transfer Latency (In seconds)		
			1st DAY	7th DAY	15th DAY
1.	I	Ethanol	25.2±2.35	28.6±3.89	28.8±4.04
2.	II	Standard+ethanol	6.6±1.47	5.6±1.21	3.6±0.68
3.	III	SLAE+ ethanol	8.4±1.69	7.6±2.42	4.2±0.97
4.	IV	SLME+ ethanol	6.8±1.62	5.4±1.54	3.8±0.66

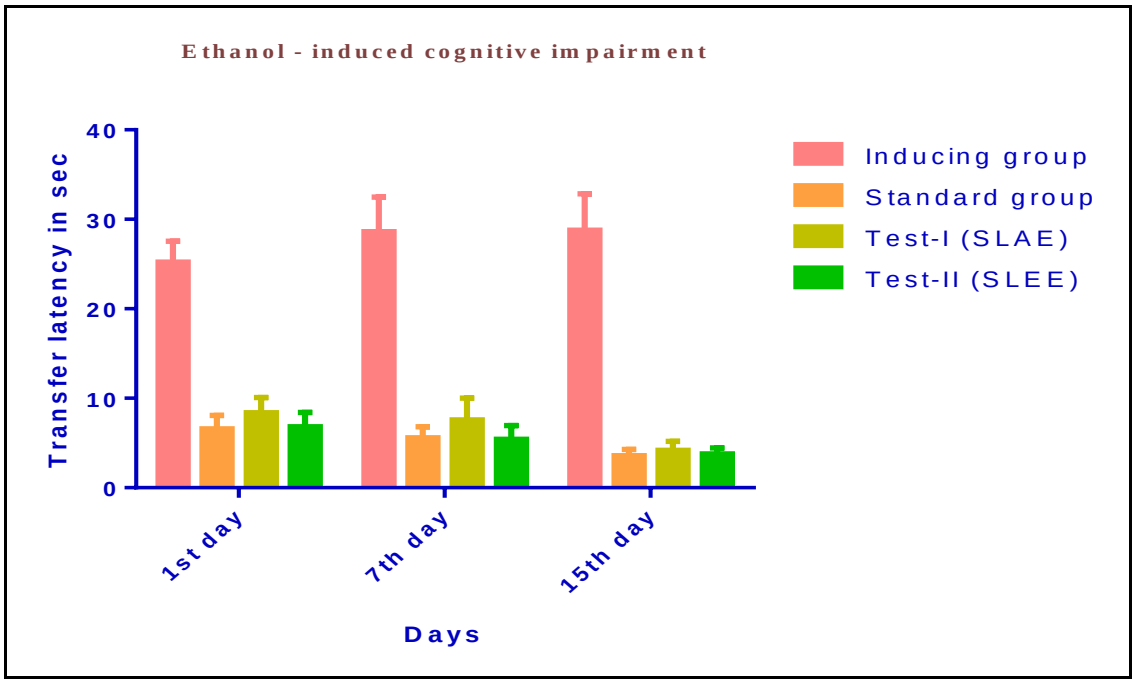

Figure 3:Effect of stem extracts of *Securinega leucopyrus* on ethanol- induced cognitive impairment. Values are expressed as Mean ± SE, p < 0.01 vs. control (n = 5 animals)

Table 5: Effect of stem extracts of *Securinega leucopyrus* on diazepam induced amnesia

S.No.	Group	Treatment	Transfer latency (in seconds)	
			8th DAY	After 24 hours (i.e.9th DAY)
1.	I	Diazepam	27.2±3.89	25.8±4.44
2.	II	Standard+ diazepam	5.0±1.14	3.0±0.32
3.	III	SLAE+ diazepam	10.6±2.4	7.6±1.12
4.	IV	SLME+diazepam	6.6±0.4	3.2±0.8

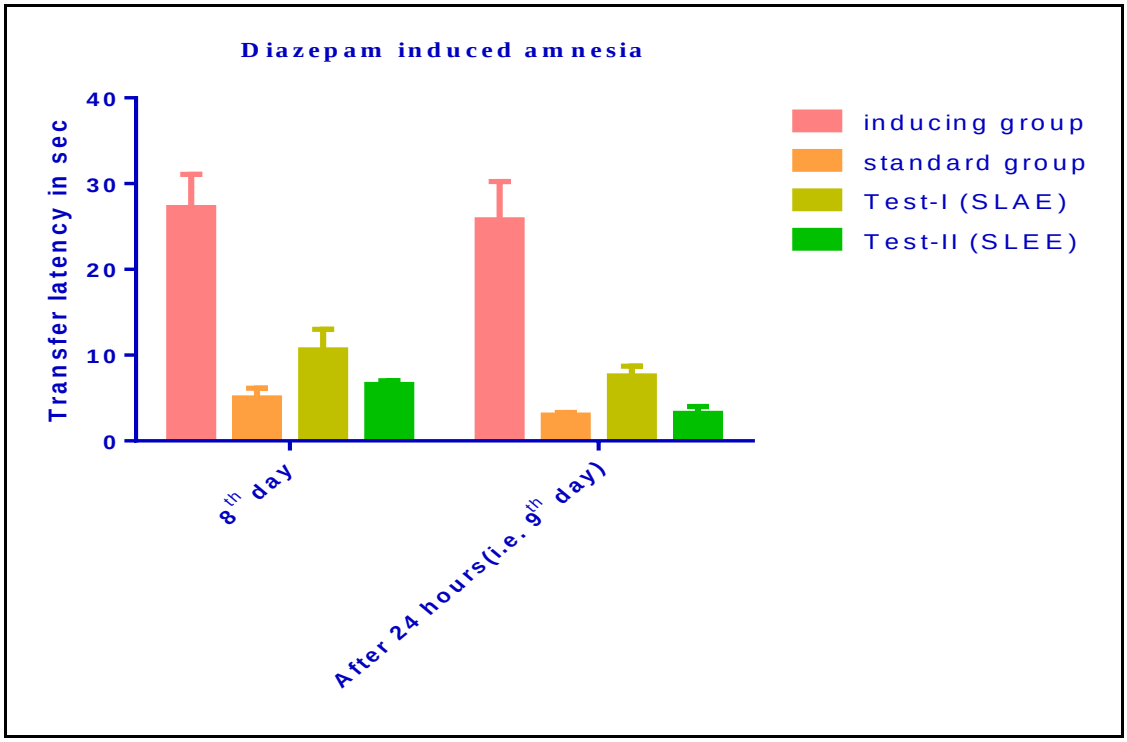

Figure-4:Effect of stem extracts of *Securinega leucopyrus* in Diazepam induced amnesia. Values are expressed as Mean ± SE, p < 0.05 vs. control (n = 5 animals)

Conclusion

The evaluation of methanolic and aqueous stem extracts of *Securinega leucopyrus* has shown significant activity towards Alzheimer's disease. The formulation and further research on *Securinega leucopyrus* may give a valuable resultsdue to the presence of saponins and flavonoids. The anti-oxidant activity of *Securinega leucopyrus* gives hope for the research related to the invitro activities in the area of preclinical research.

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