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Study on nootropic activity of alcoholic extracts of flower of *Securinega leucopyrus* (AEFSL) in mice

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ABSTRACT

To evaluate the notropic activity of alcoholic extract of flower of *Securinega leucopyrus* (AEFSL) using Interoceptive Behaviour model in mice. Nootropic activity of the AEFSL was evaluated using Interoceptive Behaviour models (Diazepam and scopolamine induced amnesia) in mice, Standard drug like Piracetam was used as reference in each model. Preliminary phytochemical studies with AEFSL revealed the presence of phytoconstituents like glycosides and flavonoids in the extract. When this extract is subjected for LD₅₀ studies, produced abnormal behaviour or mortality even at the dose level of 2000 mg/Kg body weight in mice. Three different doses like low 1/20th (100 mg/Kg), medium 1/10th (200 mg/Kg) and high 1/5th (400 mg/Kg) doses from the maximum dose tested for LD₅₀ were selected for the present study. Piracetam and AEFSL treated groups when compared to Diazepam and scopolamine induced amnesic mice the increased inflexion ratio were observed. The present study on notropic activity with AEFSL confirmed the above mentioned effect because of several phytoconstituents like flavonoids as these were already reported for their nootropic activity.

Keywords: *Securinega leucopyrus*, Flower, Alcoholic extract, Diazepam, Scopolamine, nootropic, and Piracetam.

INTRODUCTION

Nootropics, popularly referred to as “smart drugs” are substances, which boost human cognitive abilities. Typically these are alleged to work by increasing the brain’s supply of neurochemicals, improving brain’s oxygen supply or by stimulating nerve growth.¹

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 of life style changes for controlling age related neurodegenerative disorders². Alzheimer’s disease (AD) is characterised by degenerative changes in the brain accompanied by loss of memory, especially for recent events. The learning and memory is closely associated with the functional status of the central cholinergic system. The basal forebrain provides the major source of cholinergic inputs to the neocortex and hippocampus. The main cholinergic pathways in the mammalian forebrain

are the projection from the medial septal nucleus and the nucleus of the vertical limb (diagonal band of Broca) to the hippocampus via the fimbria-fornix and the projection from nucleus basalis cell ularis to the neocortex³ Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, people are now motivated to explore the Indian traditional system to come up with a promising solution to manage this deadly disease (AD).

This plant contains flavonoids, alkaloids, glycosides, tannins, sterols, triterpenes^[4]. There are reports showed that leaves possess antioxidant, antiinflammatory, antipyretic, antiarthritic and antimicrobial activity^[4, 5, 6]. Paste of the leaves is used along with tobacco to destroy worms in sores^[7]. Boiled leaves are eaten twice a day for dysentery and for dandruff^[8]. In the ethno medicinal plant survey, Pradip Bhat et al, 2013, indicated its use in boils and Eczema^[9]. Donda et al., 2013 synthesized silver nanoparticles using extracts of *Securinega leucopyrus* and evaluated its antibacterial activity^[10]. Traditional practitioners are using it to prevent miscarriage and misconception^[11]. The present study was focused upon exploring the potential of an Indian medicinal plant, "*Securinega leucopyrus*" for its efficacy in reversing the memory deficits and for its improving acquisition and memory retention in experimental

MATERIALS AND METHODS

1.1. Plant Collection

The aerial part of the plant *Securinega leucopyrus* was collected locally, from the moist region Vainganga River, Bhandara[MS, India]. The sample of plant was identified and authenticated by Dr. Jagannath Gadpayle, Botanist, S. N. Mor College of Science, Tumsar, Bhandara [MS] India.

1.2. Extraction

Freshly collected aerial parts (fruits) of the plant *Securinega leucopyrus* were washed, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and weighed quantity of the powder (800 g) was subjected to hot percolation in a Soxhlet apparatus using ethanol, at a temperature range of 40-80⁰C. The marc was completely dried and weighed. The Alcoholic extract of fruits of *Securinega leucopyrus* (AEFSL) was concentrated to a dry mass by concentrating on water bath and keeping it in desiccators^[12].

1.3. Preliminary Phytochemical Test

The defatting is done by petroleum ether. Ethanolic extract obtained by the above methods from *Securinega leucopyrus* (AEFSL) were subjected to qualitative test for the identification of various metabolites of plant by the standard procedures^[13].

1.4. Experimental Animals

Albino mice of either sex weighing between 18-22 were obtained from the animal house of Shree Farms, Nimgaon, Pahela, Bhandara, India [1231/b/08/CPCSEA] and used for present study. The animals were housed in groups of six per polypropylene cages and maintained at 24⁰C ± 1⁰C with the relative humidity of 45-55 % and 12:12 h dark light cycle. The experiments were carried out between 10:00 to 17:00 h. The animals had free access to food (standard chew pallets, Trimurti foods, Nagpur, India) and water ad libidum. The institutional animal ethics committee [928/ab/06/CPCSEA] of Manoharbhay Patel Institute of Pharmacy, Kudwa, Gondia, (MS), India approved the Pharmacological and acute toxicity protocol.

1.5. Determination of LD₅₀ of AEFSL

The acute toxicity of the extract was determined by using albino mice of either sex (18-22 g) maintained under standard husbandry conditions were fasted for 3 h prior to the experiment. Animals were administered with single doses of either AEFSL and observed for their mortality up to 48 h study period (short term toxicity). Based on the short-term toxicity profile, the next dose of each extract was determined as per OECD Guidelines No 425. From the LD50 dose of individual extracts 1/20, 1/10 and 1/5th doses selected and considered as low, medium and high dose respectively and were used in the entire study¹⁴.

2.6 Experimental protocol

2.6.1. Diazepam induced amnesia in mice (Interoceptive Behaviour model)

All groups were treated respectively as mentioned below for a period of 15 days and Diazepam 1 mg/kg was given i.p 90 min after the last dose of standard/ AEFSL to induce impairment of memory that act through GABAergic system. Transfer latency (TL) was recorded with Elevated Plus maze (EPM) at 45 min and 24 h after injection of

Diazepam. The inflexion ratio was calculated by the following formula: $IR = (L_0 - L_t) / L_0$ Where L_0 – initial transfer latency on the 15th day. L_t – Transfer latency on 16th day^{15,16}.

Treatment schedule

Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Group II with Diazepam alone (1mg/kg, i.p) only on 15th day, Group III with piracetam (200 mg/kg, p.o.) which served as standard and Groups IV, V, VI and, were treated with different doses of AEFSL (100,200 and 400 mg/kg p.o.) respectively and after 90 min of the last dose for all the groups from III, IV, V, VI were given with Diazepam (1 mg/kg, i.p).

2.6.2. Scopolamine induced amnesia in mice (Interoceptive Behaviour model)

All groups were treated respectively as mentioned below for a period of 15 days and Scopolamine 1 mg/kg was given i.p 90 min after the last dose of standard/ AEFSL to induce impairment of memory that act through GABAergic system. Transfer latency (TL) was recorded with Elevated Plus maze (EPM) at 45 min and 24 h after injection of Diazepam. The inflexion ratio was calculated by the following formula: $IR = (L_0 - L_t) / L_0$ Where L_0 – initial transfer latency on the 15th day. L_t – Transfer latency on 16th day^{15,16}.

Treatment schedule

Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Group II with Scopolamine alone (1mg/kg, i.p) only on 15th day, Group III with piracetam (200 mg/kg, p.o.) which served as standard and Groups IV, V, VI and, were treated with different doses of AEFSL (100,200 and 400 mg/kg p.o.) respectively and after 90 min of the last dose for all the groups from III, IV, V, VI were given with Scopolamine (1 mg/kg, i.p).

Table No. 1. Effect of AEFSL on inflexion ratio in Diazepam induced amnesic model in mice

Treatment	Dose/kg	Inflexion Ratio(mean±sem)
Normal control(vehicle)	10 ml p.o	0.5085 ±0.013
D IAZEPAM alone	1 mg i.p	0.309167 ±0.041
Piracetam + Diazepam	200MG I.P + 1mg i.p	0.684333 ±0.014**
AEFSL+ Diazepam	100MG I.P + 1mg i.p	0.4165 ±0.023*
AEFSL+ Diazepam	200MG I.P + 1mg i.p	0.581667 ±0.031**
AEFSL+ Diazepam	400MG I.P + 1mg i.p	0.653167 ±0.035**

Effect of AEFSL on IR in Diazepam induced amnesic model in mice

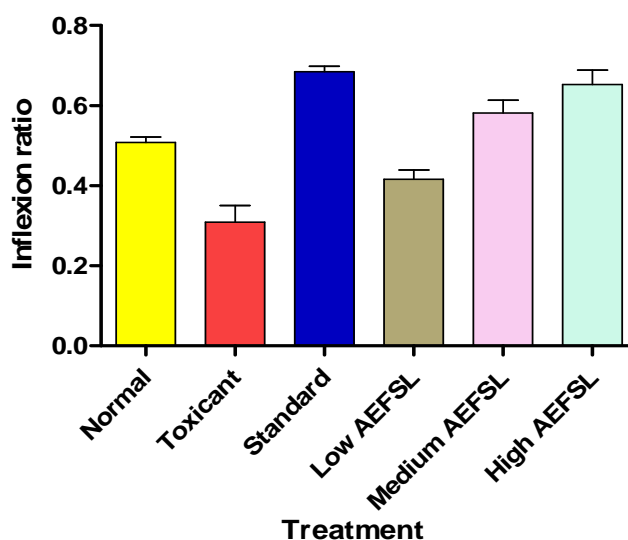
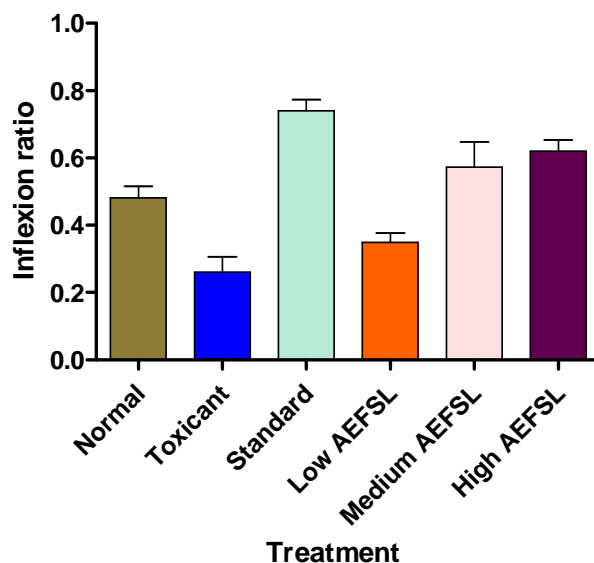


Table No. 2. Effect of AEFSL on inflexion ratio in Scopolamine induced amnesic model in mice

Treatment	Dose/kg	Inflexion Ratio(mean±sem)
Normal control(vehicle)	10 ml p.o	0.481 ±0.034
Scopolamine	1 mg i.p	0.260833 ± 0.045
Piracetam +Scopolamine	200MG I.P + 1mg i.p	0.7405± 0.032**
AEFSL+ Scopolamine	100MG I.P + 1mg i.p	0.349167±0.028*
AEFSL+ Scopolamine	200MG I.P + 1mg i.p	0.572667±0.075**
AEFSL+ Scopolamine	400MG I.P + 1mg i.p	0.620533±0.033**

Effect of AEFSL on IR in Scopolamine induced amnesic model in mice



1.6. 3. Statistical analysis

The results are expressed as mean±SEM, (N=6). Statistical significance was determined by one-way analysis of variance with $P < 0.05$ considered significant. The analysis was performed by Graph Pad Prism software.

RESULTS

Effect of AEFSL on inflexion ratio in mice (Diazepam-induced amnesic model)

When compared to normal control IR 0.5085 ± 0.013 , Diazepam has induced dose dependent amnesia and in this amnesic model. When compare to toxicant group a decrease in IR 0.309167 ± 0.041 was observed. Piracetam has shown a significant increase in the IR 0.684333 ± 0.014 AEFSL with Low, medium and high dose treated groups have shown significant increase in the IR 0.4165 ± 0.023 , 0.581667 ± 0.031 , 0.653167 ± 0.035 . Results are reported in Table 1.

Effect of AEFSL on inflexion ratio in mice (Scopolamine-induced amnesic model)

When compared to normal control IR 0.5085 ± 0.034 , Scopolamine has induced dose dependent amnesia and in this amnesic model. When compare to toxicant group a decrease in IR 0.309167 ± 0.045 was observed. Piracetam has shown a significant increase in the IR 0.684333 ± 0.032 AEFSL with Low, medium and high dose treated groups have shown significant increase in the IR 0.58325 ± 0.028 , 0.568333 ± 0.075 , 0.675083 ± 0.033 . Results are reported in Table 2.

DISSCUSION

The elevated plus maze is used to measure the anxiety state in animals, however transfer latency (TL) i.e. the time elapsed between the movement of the animal from an open arm to an closed arm will be markedly shortened if the

animal had previously experienced entering from open arm to closed arms and this shortened transfer latency has been shown to be related with memory processes. In EPM acquisition (learning) can be considered as transfer latency on 1st day trials and the retention/ consolidation (memory) is examined 24 h later¹⁷. The animals shown a significant decrease in transfer latency as compared with the normal control group, which is an indication of the enhanced cognitive effect of all doses of AEFSL except 100 mg/kg and piracetam in mice. The impairment of learning and memory induced by scopolamine (1.0 mg/kg) as an anticholinergic agent was reflected by prolonged TL from the open arm to the closed arm i.e., decreased IR was observed with EPM.¹⁸ The all doses of AEFSL except 100 mg/kg and piracetam have reversed the amnesia induced by scopolamine, i.e. decreased TL from the open arm to the closed arm i.e., increased IR, indicates that extracts acting on Ach receptors because they have shown nootropic activity in presence of scopolamine which is a muscarinic receptor antagonist. Diazepam, a GABA mimetic agent induces memory impairment and the subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory.¹⁹ Diazepam (1mg/kg) has prolonged TL from the open arm to the closed arm i.e., decreased IR. The all doses of AEFSL except 100 mg/kg and piracetam have decreased TL from the open arm to the closed arm i.e., increased IR thus confirms their nootropic activity. The protective effect offered by except 100 mg/kg and Piracetam against diazepam- induced amnesic model may be due to indirect release of Ach in the brain.

CONCLUSION

From the study, it is concluded that AEFSL shows nootropic activity. Increase in dose of AEFSL result in increase in the nootropic activity of AEFSL. Hence drug is showing dose dependant activity.

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