Investigation on Learning and Memory Enhancing activity of Essential Oil in *Albizia julibrissin* Flowers in Experimental Mice

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ABSTRACT :
Two doses (100 mg/kg and 200 mg/kg p.o) of the *Albizia julibrissin* essential oil (AJEO) were subjected for the evaluation of learning and memory enhancing activity against amnesia induced by scopolamine (0.4mg/kg, i.p) in young mice. Piracetam (400 mg/kg i.p) was served as standard in both the models. Biochemical parameter like Anticholinesterase activity was evaluated. Both the lower (100mg/kg) and higher dose (200mg/kg) of AJEO has shown dose dependent significant decrease in Transfer latency (TL) by EPM and decrease in AChE activity in brain estimation which in terms indicate improved learning and memory when compared with scopolamine group. Sub-acute treatment (long term) was more significant than acute treatment (short term) on learning and memory enhancing activity.

Keywords: Cognition, Dementia, Learning and memory, AChE activity.

INTRODUCTION:

Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as cognition[1]. Memory is a fundamental mental process and without it we are capable of nothing. It is a faculty by which sensations, impressions, and ideas are stored and recalled. Learning and memory is one of the most intensively studied subjects in the field of neuroscience[2]. Dementia is a syndrome caused by disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgement[3].

Aging demographic transition is proceeding rapidly especially in India, China, and Latin America, where dementia is rapidly becoming the major public health problem[4]. Approximately 10% of the adults older than 65 years, and 50% of the adults older than 90 years have dementia[5]. Nootropic agents such as piracetam, pramicacetam, aniracetam and choline esterase inhibitors like donepezil are being primarily used to improve memory. However, the resulting adverse effects associated with these agents such as hepatotoxicity, nasal congestion, hypotension, gastro intestinal disturbances, rashes, constipation, tiredness, headache, drowsiness and systemic side effects upon chronic use have limited their use[6].

Albizia julibrissin is a "Plant for future", commonly known as mimosa. It is also known by name "Silk Tree"[7]. *Albizia* is traditionally known in China as "Happiness Herb". It has been used for centuries in "Chinese traditional herbalism" for its 'mood supportive' and 'calming properties'[8], also for depression and anxiety[7].The antianxiety, antidepressant, anti-angiogenic, anti-tumor and sedative properties of the plant were scientifically proved[9].

MATERIALS AND METHODS

ANIMALS:

The healthy albino mice (18-20g) of either sex used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore.The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

PLANT MATERIAL:

The fresh flowers of *Albizia julibrissin* used for the present

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studies were collected from Mangalore, Karnataka in July 2015. It was authenticated by Mr. Dinesh Nayak, Advisor (Green belt), Mangalore SEZ Limited. The flowers were dried under shade. The dried flowers were ground in to a coarse powder in an electrical blender and were used for extraction.

EXTRACTION OF ESSENTIAL OIL[10]:

Essential oils of tested material were obtained by the of the powdered flower samples (200g) using a simple steam distillation apparatus, for 3 hours at 100°C. EOs were dried over anhydrous sodium sulphate, filtrated, and stored in a freezer until used.

DRUGS AND CHEMICALS:

Chemicals like anhydrous sodium sulphate, Chloroform, Sodium hydroxide of pure analytical grade were procured from E. Merck (India) Ltd, Mumbai and drugs like piracetam were obtained from local suppliers. Scopolamineand5,5'Dithio nitro benzoic acid (DTNB) were procured from Himedia.

PREPARATION OF STOCK SOLUTION OF THE EXTRACT FOR DOSING:

All the doses of Essential Oil of *Albizia julibrissin* were suspended in 5% tween 80. Each time fresh preparation of the extract was prepared before administration. AJEO was administered post orally at a constant volume of 10 ml/kg for each animal. Acute toxicity studies were carried out according to OECD guidelines Doses were estimated as low dose 100mg/kg and high dose 200mg/kg.

ASSESSMENT OF NOOTROPIC ACTIVITY:

Most of the currently used paradigms for learning and memory can be conveniently discussed under behavioral tasks: Behavior on mazes (ex: Elevated Plus Maze and Morris Water Maze). Mazes are the traditional tool in assessing learning and memory performance in laboratory animals.

ELEVATED PLUS MAZE[11]:

The elevated plus maze served as the simple behavioral model which has been used from decades to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) facing each other with an open roof. A fine white line may be drawn in the middle of the floor of each enclosed arm. The arms extended from a central platform (5 cm × 5 cm) and maze was elevated to a height of 25 cm from the floor. In this model the change in the latency to go from open arm to closed arm of the elevated plus maze is an indicator of learning and memory. On the first day each mice were placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was the time taken (in sec) by the animal to move from the open arm into any one of the enclosed arms with all its four legs.

EXPERIMENTAL DESIGN:

Mice of either sex weighing between 18-20g were divided into 5 groups of six animals each.

Treatment

Group I : Animals served as control and were received only vehicle.

Group II : Animals were administered with Scopolamine, (0.4 mg/kg i.p.)on 19th& 27th.

Group III : Animals were treated with standard drugPiracetam (400mg/kg i.p.)

Group IV : Animals were treated with low dose of *Albizia julibrissin* (100mg/kg p.o) for 27 days + Scopolamine, (0.4 mg/kg i.p.) on 19th& 27th.

Group V : Animals were treated with high dose of *Albizia julibrissin* (200mg/kg p.o) for 27 days + Scopolamine, (0.4 mg/kg i.p.) on 19th&27th.

PROCEDURE:

From day 1-15 Animals were administered with drugs as mentioned above. Ondays 16, 17 and 18, animals were trained thrice daily on Elevated plus Maze (EPM); and drug administration is continued. The present study for assessment learning and memory had been split into 2 intervals. From day 1 to 20th acute study (short term) and from day 21st to 28th sub-acute study (long term) was been performed.On day 19, scopolamine (0.4 mg/kg i.p.) was administered to all animals (except group I) 30 min after the respective treatment, after 45 min assessment of final TL (transfer latency) was done using EPM. To find out the retention of memory, 24 hours later once again TL was taken down.The respective treatments with drugs were continued for next 1 week. (Days 20-26). On day 27, scopolamine 0.4 mg/kg i.p. was injected to all the animals, 30 min prior to the last drug treatment. Assessment of learning was recorded after 45 min using EPM. To find out the retention of memory, 24 hours later once again TL was taken down. TL was recorded which indicates memory.

BIOCHEMICAL ESTIMATION

Estimation of brain AChE activity[12]:

On day 28th following the behavioral testing, animals were sacrificed and the brain tissues were quickly removed, cleaned with ice-cold saline and stored at –80°C for biochemical estimation.

Animals were sacrificed by cervical dislocation immediately after the behavioral analysis. Decapitated and removed the brain and 10% (w/v) tissue homogenate was prepared in chilled phosphate buffered saline (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10 min at 40 C to remove nuclei and unbroken cells. The pellet was discarded and a portion of supernatant was again centrifuged at 12,000 rpm for 20 min to obtain post-mitochondrial supernatant. The post-mitochondrial supernatant thus obtained was used to assay (Acetylcholinesterase, reduced glutathione, nitrite level etc.) parameters.

Acetylcholinesterase activity was measured by the method of Ellman.A photometric method for determining acetyl cholinesterase activity of tissue extracts has been described. The substrate used in the assay system wasAcetylthiocholine, the ester of thiocholine and acetic acid.0.4 ml of Supernatant Brain Homogenate was incubate for 5 min with 2.6 ml 0.05 M Phosphate Buffer and 100 micro liters (0.1 ml) 0.1M DTNB. Measure the absorbance at 412 nm until the reading is constant. Now add 20 micro liters (0.02ml) Acetylthiocholine Iodide (Substrate). Immediately take the absorbance at 412 nm for continue 7

minutes at the interval of every minute.AChE activity was calculated using the following formula:

$R=\delta$OD×volume of assay (3 ml)/E×mg of protein,

Where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. δ OD is the change in absorbance per minute and E is the extinction coefficient, which is 13 600 M^{-1} cm^{-1}.

RESULTS

ELEVATED PLUS MAZE

The results of EPM are given in (Table 1). AJEO low dose (100 mg/kg) and high dose (200 mg/kg) were administered for 27 days orally have shown significant effect on TL. High dose produced high significance ($p<0.01$) and low dose produced slight significant effect ($p<0.05$) when compared with scopolamine induced amnesic group, on 19th day and 27th day. The young animals treated with low and high dose showed remarkable reduction in TL of 19th day as well as 27th day as a part of learning and remarkable reduction in TL of 20th day and 28th day as a part of retention (memory). Altogether it indicates significant improvement in learning and memory (Fig 2).Scopolamine (0.4 mg/kg, i.p.) injected before training significantly increased TL on days 19th and 27th indicating impairment in learning and memory. Piracetam (used as standard) at a dose of 400 mg/kg (i.p.) also shown gradual decrease in TL. The effect produced by piracetam was highly significant ($p<0.001$).Sub-acute study has shown more effectiveness on learning and retention as compared with acute study. Even though both the two doses of AJEO produced significant reduction in TL, high dose was more effective than low dose.

TABLE NO 1: Transfer Latency of young mice by EPM task during learning and retention (memory) on 19th& 27th day:

Group No.	Treatment	Acute study TL (sec) mean ± S.E.M [n=6]		Sub-acute study TL (sec) mean ± S.E.M [n=6]	
		19th day	After 24 hrs.	27th day	After 24 hrs.
I	Control	33.65±0.63	30.41±0.46	24.89±0.34	21.17±0.36
II	Scopolamine 0.4 mg/kg	51.58±0.49	50.09±0.95	48.59±0.35	47.46±0.85
III	Scopolamine 0.4 mg/kg + Piracetam 400 mg/kg	36.55±0.51***	32.55±0.31***	28.47±0.54***	24.37±0.24***
IV	Scopolamine 0.4 mg/kg + AJEO 100 mg/kg	42.57±0.87*	39.87±0.17*	34.64±0.86*	30.66±0.76*
V	Scopolamine 0.4 mg/kg + AJEO 200 mg/kg	39.62±0.58**	35.52±0.22**	31.15±0.61**	27.25±0.43**

Mean latencies (learning &memory scores) across 19th& 27th day in the EPM task; n=6; Values are expressed as Mean ± SEM; * P<0.05, ** P<0.01, *** P<0.001 compared to scopolamine group. (ANOVA followed by Dunnett's multiple comparison test) AJEO: *Albizia julibrissin* Essential Oil

ESTIMATION OF ACETYL CHOLINESTERASE ENZYME ACTIVITY

The results and the statistical analysis for the changes in acetylcholine esterase (AChE) concentration in isolated mice's whole brain homogenate after administration of the essential oil of *Albizia julibrissin* are given in (TABLE 2). The low dose of AJEO 100 mg/kg (p.o) produced slight significance ($p<0.05$) and high dose 200 mg/kg (p.o) produced highly significant effect ($p<0.01$) on cholinesterase activity in young mice as compared to control group by using Ellman's kinetic colorimetric method. In the control group receiving only vehicle, the AChE concentration was found to be lower than that of the amnesia induced mice (Fig 2). The AChE content of whole brain homogenate was decreased by AJEO in dose dependent manner. High dose long term administered group was more effective than low dose, when compared to control group indicates effectiveness for learning and retention.

Table No 2: Effect of AJEO on Acetylcholine esterase (AChE) activity

Group No.	Treatment	Dose	AChE (µ moles) mean ± S.E.M
I	Control	10 ml/kg	20.90±1.64
II	Scopolamine	0.4 mg/kg (i.p)	26.38±2.55
III	Scopolamine + Piracetam	400 mg/kg (i.p)	19.16±0.93 ns
IV	Scopolamine + AJEO low dose	100 mg/kg (p.o)	14.26±2.34*
V	Scopolamine + AJEO high dose	200 mg/kg (p.o)	10.69±0.98**

n=6; Values are expressed as Mean ± SEM; * p<0.05, ** P<0.01, ns: not significant compared to control group. (ANOVA followed by Dunnett's multiple comparison test). AJEO: *Albizia julibrissin* Essential Oil

DISCUSSION:

Nootropic agents or cognition enhancers are few synthetic medicines e.g. tacrine, donepezil and the natural product based rivastigmine for treatment of cognitive dysfunction and memory loss associated with dementia. Reported to have adverse effects including gastrointestinal disturbances and problems associated with bioavailability, which necessitates the interest in finding better from natural resources[13].

Cholinergic neurons originating in the medial septum project to areas such as the cortex and hippocampus, which

play a role in Ach-associated cognition. For many years, the amnesic action produced in animals by the administration of centrally acting muscarinic cholinergic antagonists, particularly scopolamine, has been a widely used model for the characterization of potential cognition-enhancing drugs. Scopolamine induced amnesic rodent model is one of the well-established animal model for memory dysfunction[14]. Scopolamine-induced amnesia was proposed to be due to blockade of cholinergic neurotransmission, this substance is used to model the cognitive deficits that could be observed in dementia. Systemic administration Scopolamine induces central cholinergic blockade, produced a reversible and well described impairment in both (i) maintaining attention and (ii) processing of information and the acquisition of new knowledge in rodents and in humans.

Piracetam, the established nootropic agent was used in the present study as standard because; it improves memory by facilitation of synaptic transmission (increase choline uptake in cholinergic nerve endings, thereby facilitating cholinergic transmission) in brain[15].

The EPM served as simple behavioral model to evaluate learning and memory in mice, is a widely accepted paradigm to study learning and memory processes in rodents[11]. In the present study AJEO improved the memory retention of mice as reflected by diminished transfer latency (TL) values on EPM. The TL is an index used for assessment of memory retention potential.

ACh is considered as the most important neurotransmitter involved in the regulation of cognitive functions. As dementia is characterized by low levels of the neurotransmitter, ACh in brain, it can be treated by enhancing cholinergic function by stimulation of cholinergic receptors or prolonging the availability of ACh released into the neuronal synaptic cleft by use of agents which restore or improve the levels of ACh.

Essential oils carry biologically active volatile compounds in a highly concentrated form that can provide therapeutic benefits in very small amounts. The fragrance also affects the limbic system in brain, which controls both memories and emotions. Inhaling the fragrance of an essential oil, the aroma tends to penetrates bloodstream via lungs, and this is thought to be one of the mechanisms by which aromatherapy exerts its physiological effects. Investigation carried out on floral essential oil composition of *Albizia julibrissin* proved that the 'major components' of hydro distilled floral essential oil contains palmitic acid (23.3%), *trans*-linalool oxide (furanoid form, 6.6%,pyranoid form, 7.0%), pentacosane (7.2%), eugenol (6.1%), and 1-octanol (5.2%). Other compounds likely contributing to the fragrance were linalool (3.1%), and *cis*-linalool oxides (furanoid and pyranoid forms, 0.9% and 0.5%, respectively)[16]. Several researches on nootropic activity proved that various constituents obtaining numerous benefits on learning and memory are poly phenols[17](eugenol)[18], terpenes[19](linalool)[20].

AJEO showed decreased AChE level in mice brain, probably by inhibiting brain AChE activity in terms by inhibiting AChE enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter ACh in the central cholinergic pathways. The activity may be attributed to presence of, "polyphenolic compounds" like "eugenol", terpenes namely "linalool" and other 'antioxidant constituents' in the plant. The role of antioxidants in preventing oxidative stress-induced memory deficits is well established. Eugenol and linalool[20] has been known to demonstrate neuroprotective action possess antioxidant properties as well. Since 'eugenol and linalool' constitutes the 'major component of AJEO', it can be possible that AJEO too exhibits such antioxidant potential and modulates memory and learning processes.

Further study is needed to find its potential use in humans. The exact mechanism of action of AJEO on learning and memory enhancing activity is not known. However, further studies are necessitated to identify the exact mechanism of action.

CONCLUSION:

Administration of AJEO prevented scopolamine induced experimental amnesia and may be a great potential in memory deficits, nevertheless more studies are required to elucidate exact memory improving mechanisms of the plant. The results of the investigation justify the folklore use of *Albizia julibrissin* and the plant is worth for further chemical and pharmacological investigations.

Figure No: 1: Effect of AJEO on Transfer Latency in young mice by EPM on 19th& 27th day

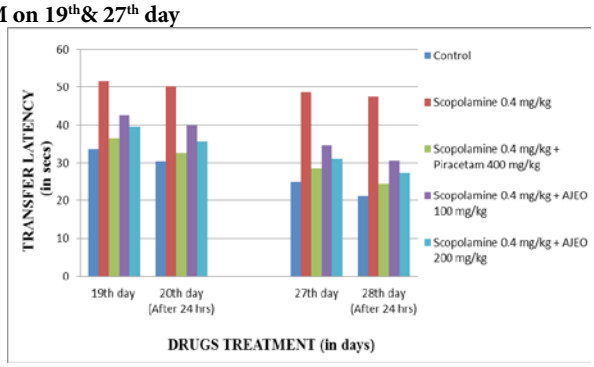
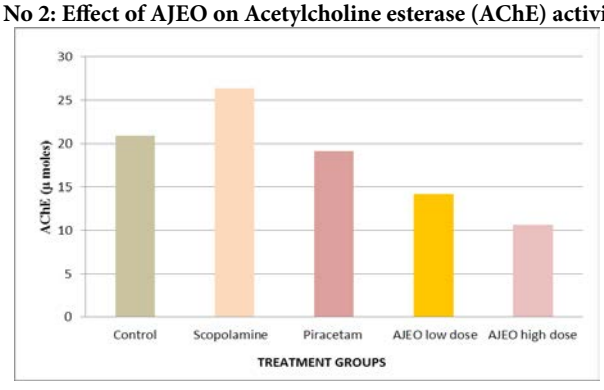


Fig No 2: Effect of AJEO on Acetylcholine esterase (AChE) activity



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