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Pharmacological evidences for the anti-alzheimer potentials of *Sesbania grandiflora Linn.* in mice

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ABSTRACT

Alzheimer's disease(AD) is characterized as a progressive neurode generative disorder and considered as prominent cause of senile dementia in the elderly. It has become recognised as a major cause of morbidity and mortality in the ageing population worldwide. The neuropathologic hallmarks of AD are neuritic plaques and neurofibrillary tangles. The present study was undertaken to investigate the anti-alzheimer's potential of *Sesbania grandiflora* Linn. (SG) in mice. Ethanolic extract of leaves and aqueous extract of flowers of SG (50 and 100mg/ kg, p.o.) were administered for 5 successive d to both young and aged mice. Exteroceptive behavioral model employed to evaluate learning and memory were elevated plus maze and interoceptive behavioral model were scopolamine (0.4mg/kg, i.p.) and natural ageing induced amnesia. Piracetam (200mg/kg, i.p.) was used as a standard nootropic agent. SG (50 and 100mg/kg, i.p.) significantly decreased whole brain acetyl cholinesterase activity, lowered serum cholesterol levels and reversed the amnesia induced by both scopolamine (0.4mg/kg, i.p.) and natural aging. Hence, SG can be a suitable memory restoring agent in the management of cognitive dysfunctions such as dementia and AD.

KEYWORDS

Dementia;
Acetylcholine;
Sesbania grandiflora;
Piracetam;
Scopolamine.

INTRODUCTION

Alzheimer's disease(AD) is a major neurodegene rative disorder affecting approximately 5% of the over 65-year old populations, but the cause of AD remains largely unknown. Currently, 4.5 million individuals in the United States are estimated to have AD, and that number is projected to increase to at least 14 million by the year 2050[1]. Neurodegeneration results from a chronic inflammatory response to deposited amyloid plaques (Aβ aggregates)[2,3], that are directly neurotoxic[4,5]. Small, soluble aggregates of Aβ peptides (Aβ oligomers) are known to alter synaptic function[6,7] and intracellular deposits of Aβ contribute to AD[8]. Increasing evidence indicates that oxidative stress plays a crucial role in the pathogenesis of idiopathic AD[9–12] and it has been associated with increased production of reactive oxygen species (ROS), which could result from a combination of aging, genetic predisposition, and environmental factors[11]. Nootropic agents such as piracetam[13], pramiracetam, aniracetam[14] and choline esterase inhibitors like donepezil[15] are useful in the re-

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duction of cognitive impairment in patients with AD, but the resulting adverse effects[16] associated with these agents have made their use limited. Hence there is a need to explore alternative remedies taking clues from traditional systems of medicine like ayurveda, Unani or tibetian medicine.

Sesbania grandiflora Linn. (Family- Fabaceae), is native of Malaysia and is widely cultivated in India. It is a small, loosely branching tree that grows up to 8-15 m tall and 25-30cm in diameter with white and red flowers. It is commonly known as agasti, agati and anari in Sanskrit; agasti, bak, basma, basna, chogache, hatiya in Hindi and Agati sesbania, August flower or tiger tongue in English. The root, stem, flower, leaf and fruits are used in Ayurveda for treating inflammation, boils, coryza, epilepsy, fever, headache, nightblindness and nervous disorders. In siddha the roots, bark, leaves and flowers are employed for treatment of food poisoning, diseases of *pittam*, worm infestation, sinusitis and opthalmic diseases[17]. It alleviates all the three doshas: *pitta, kapha*, and *visada*. It can be useful to promote intelligence, appetite and also employed in fatigue, cough, toxicosis and itching[18]. Juice of roots is given as an expectorant. Bark useful in diarrhoea, dysentery, ulceration of tongue and alimentary canal[19]. It exhibits anticonvulsant, anxiolytic[23] and haemolytic activity[24]. SG is reported to possess hepatoprotective activity[20]. It is a rich source of provitamin A carotenoid, beta-carotene[21,22], The main objective of this work was to explore the usefulness of aqueous extract of flowers and ethanolic extract of leaves on maintaining AchE, cholesterol levels and improving memory in amnesic mice.

MATERIALS AND METHODS

Collection of the plant material

The leaves and flowers of *Sesbania grandiflora* Linn.were collected from the areas of Haveri and Dharwad district, Karnataka, India, during October 2006. The plant parts were authenticated and identified by Dr. Hebbar, Department of Botany, Karnataka University, Dharwad. Voucher specimen has been deposited at Dept. of Pharmacognosy, SET'S college of Pharmacy, Dharwad, for further reference.

Preparation of extract

The leaves were cleaned, shade dried and powdered to prepare an ethanolic extract. One kilogram of finely powdered leaves of SG was extracted by refluxing with 90% ethanol in Soxhlet extractor for 15-20h. The extract was evaporated to dryness under reduced pressure and temperature using rotary vacuum evaporator. The yield of dry extract from crude powder of SG was 10% w/w. The lyophilized crystals of ethanolic extract of leaves of SG (ESG) was used to prepare a suspension using distilled water and was orally administered to animals. The aqueous infusion of SG(ASG) was prepared by soaking fresh flowers in distilled water in the ratio of 1:5(w/v) along with 10ml of ethanol for 5 d (maceration process). The menstrum was filtered and the filtrate was administered orally. The volume of administrating was 1ml/100g, body weight of mice.

Chemicals

Scopolamine hydro bromide (Sigma Aldrich, USA), piracetam (Nootropil®, UCB India Pvt. Ltd., Gujarat, India), diazepam (Calmpose®, Ranbaxy, India) and phenytoin (Zydus Neurosciences, Ahmedabad, India) were diluted in normal saline. Volume of i.p. administration was 1ml/100g body weight of mice.

Animals

Swiss mice of either sex weighing around 18g(younger, 8 weeks old) and 25g(older, 28 weeks old) were used in the present study. Animals were procured from disease free animal house, BLDEA Medical College, Bijapur. They were acclimatized to the laboratory conditions for 5 d before behavioral studies. The animals had free access to food and water and maintained under 12**:**12 h light and dark cycles. All experiments were carried out during d time from 0900 to1900h. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Ministry of Forest and Environment, Govt. of India.

Acute toxicity studies

Ethanolic and aqueous extracts of SG (50 and 100mg/kg, p.o.) were administered to normal mice. During the first four h after the drug administration, the animals were observed for gross behavioral changes if

any for 5 d. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality were observed.

Memory models

Exteroceptive behavioral model

Elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The procedure and endpoint applied in the present study for testing learning and memory was as per the criteria described by the investigators working in psychopharmacology and behavioral pharmacology[25,26]. The apparatus consisted of two open arms (16cm×5cm) and two covered arms (16cm5cm×12cm). The arms extended from a central platform (5 cm×5cm), and maze was elevated to a height of 25cm from the floor. On the 1st d, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency(TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded on the first d. If the animal did not enter into one of the covered arm within 90s, it was gently pushed into one of the two covered arms and the TL was assigned as 90s. The mouse was allowed to explore the maze for 10 s and then returned to its home cage. Memory retention was examined 24h after the 1st d trial on the 2nd d.

Interoceptive behavioral models

(a) Diazepam -induced amnesia

Diazepam (1mg/kg, i.p.) was injected intraperitoneally into young mice on fourth d and TL was recorded after 45 min of injection. Memory/retention was recorded on 5th d i.e. after 24h.

(b) Scopolamine-induced amnesia

Scopolamine (0.4mg/kg, i.p.) was injected intraperitoneally into young mice on 4th and 5th d and TL were recorded 45min after injection. Memory retention was examined after 24h (i.e. on 5th d).

Estimation of brain acetyl cholinesterase (AChE) activity

The time frame of cholinesterase activity estimation was similar to behavioral tests i.e. 8 AM- 11 AM on each d. On the 5th d the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using the Ellman method[27]. The end point was the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5'-dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow color compound formed during the reaction at 412nm every min for a period of three minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

R= δ O.D.×Volume of Assay (3ml)/ E×mg of protein

Where R=Rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/minute /mg protein, δ O.D. =Change in absorbance/min, E=Extinction coefficient = 13600/M/cm

Estimation of Serum Glucose and cholesterol levels

On the 5th d the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue , blood sample was collected and total serum glucose levels was estimated by GOD-POD method[28]. The enzymatic method, CHOD-PAP was used for the estimation of total cholesterol[29,30].

Statistical analysis

The values are expressed as mean ±S.E.M. The data were analysed using ANOVA followed by Tukey-Kramer test. $P<0.01$ was considered as stastically significant.

RESULTS

Effect on transfer latency (TL) using elevated plus maze

Transfer Latency (TL) of 2nd d (5th d of drug treatment) reflected retention of learned task or memory. The young animals treated with SG (50 and 100mg/kg, p.o.) showed dose- dependent reduction of TL on 5th d, indicating significant improvement in memory, when

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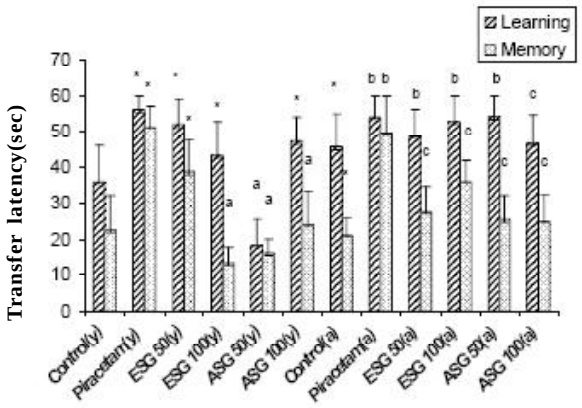
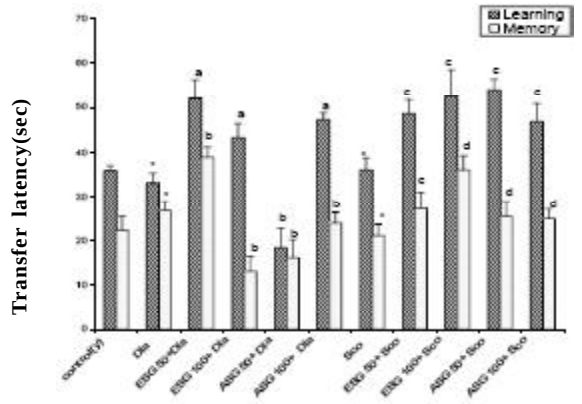


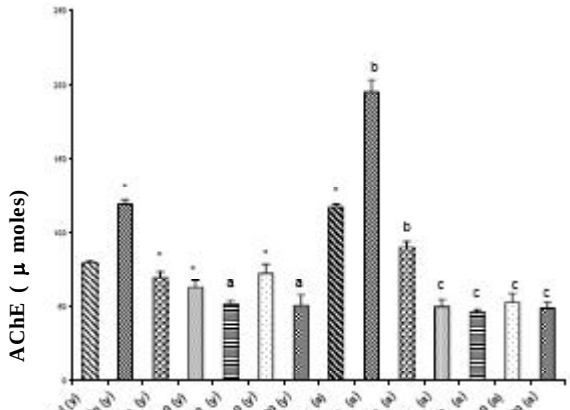
Values are mean ±S.E.M, (n=5), ANOVA follows Tukey-Kramer test; *P<0.01 compared to control (Young), [a]P<0.001 compared to control (Young), [b]P<0.01 compared to control (Aged), [c]P <0.001 compared to control (Aged).

Figure 1 : Effect of *S.grandiflora* on transfer latency of young and aged mice



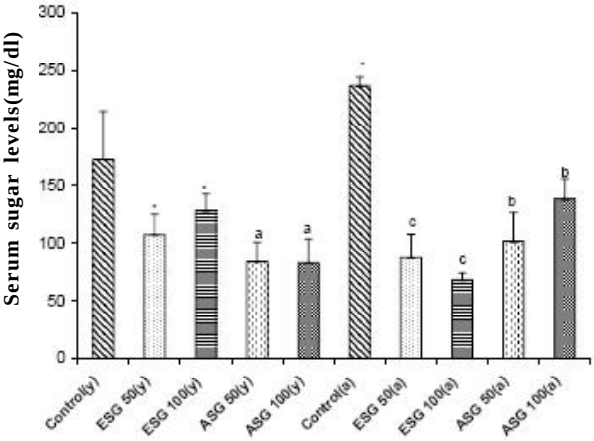
Values are mean ± S.E.M, (n = 5), ANOVA follows Tukey-Kramer test; *P<0.01 compared to control (Young), [a]P<0.01 compared to diazepam treated mice, [b]P<0.001 compared to diazepam treated mice, [c]P<0.01 compared to scopolamine treated mice, [d]P<0.001 compared to scopolamine treated mice.

Figure 2: Effect of *S.grandiflora* on TL of diazepam and scopolamine induced amnesia



Values are mean ±S.E.M, (n=5), ANOVA follows Tukey-Kramer test; *P<0.01 compared to control (Young), [a]P<0.001 compared to control (Young), [b]P<0.01 compared to control (Aged), [c]P<0.001 compared to control (Aged).

Figure 3 : Effect of *S.grandiflora* on brain AChE activity of young and aged mice.



Values are mean ± S.E.M, (n=5), ANOVA follows Tukey-Kramer test; *P<0.01 compared to control (Young), [a]P<0.001 compared to control (Young), [b]P<0.01 compared to control (Aged), [c]P<0.001 compared to control (Aged).

Figure 4 : Effect of *S.grandiflora* on serum sugar levels of young and aged mice

compared with control group. SG (50 and 100mg/kg, p.o.) also produced significant improvement in memory (P<0.01) of older mice (Figure 1). Scopolamine (0.4mg/kg, i.p.) and diazepam (1ml/kg, i.p.) injected before training significantly increased (P<0.01) the TL of 4th and 5th d indicating impairments in learning and memory (amnesia). The mice treated with SG (50 and 100mg/kg, p.o.) for 5 successive d) reversed successfully the amnesia induced by scopolamine, diazepam and natural ageing (Figure 2). Piracetam (used as the positive control) at the dose of 200 mg/kg, i.p. improved memory (P<0.01) of both young and older mice and reversed the amnesia induced by scopolamine and diazepam.

Effect on whole brain acetylcholinesterase activity

The whole brain AChE activity with phenytoin (12mg/kg, p.o.) demonstrated significant rise in AChE activity as compared to control and piracetam (200mg/kg, i.p.). SG (50 and 100mg/kg, p.o.) significantly (P<0.001) lowered whole brain AChE activity (Figure 3).

Effect on Serum Glucose levels

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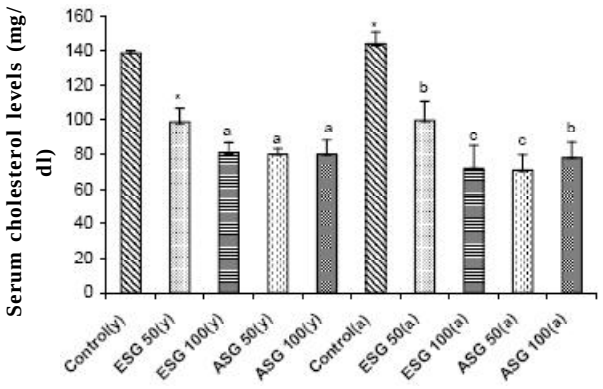

Values are mean ± S.E.M, (n= 5), ANOVA follows Tukey-Kramer test; *P<0.01 compared to control (Young), [a]P<0.001 compared to control (Young), [b]P<0.01 compared to control (Aged), [c]P<0.001 compared to control (Aged).

Figure 5 : Effect of *S.grandiflora* on serum cholesterol levels of young and aged mice

Ethanolic and aqueous extract of SG (50 and 100mg/kg, p.o.) did not show any significant change in total serum glucose levels of aged mice. The animals receiving ethanolic and aqueous extract of SG (50 and 100mg/kg, p.o.) for 5 d consecutively showed significant reduction in total serum glucose levels of young (P<0.001) mice, when tested using Autoanalyzer (Figure 4).

Effect on Serum Cholesterol levels

The animals administered with ethanolic and aqueous extract of SG (50 and 100mg/kg, p.o.) for 5 d consecutively showed significant reduction in total serum Cholesterol levels of (P<0.001) mice, when tested using Autoanalyzer (Figure 5). when compared with that of control.

CONCLUSION

There has been a steady rise in the number of patients suffering from Alzheimer's disease(AD) all over the world. Alzheimer's disease(AD) is characterized histopathologically by accumulation of amyloid plaques and neurofibrillary tangles with amyloid-β peptide(Aβ) as a major component of AD-related plaques. Numerous evidence indicates that different forms of Aβ aggregates play an important role in AD pathogenesis [31,32]. The deposition of intracellular aggregates containing abnormally phosphorylated forms of the microtubule-binding tau protein is another characteristic feature[33]. Tau protein is predominantly expressed in axons, where it binds to and stabilizes microtubules [34], and is also the main component of paired helical filaments (PHFs). PHFs form neurofibrillary tangles(NFTs) that are a pathological feature of Alzheimer's disease (AD) [34,35] and several other "tauopathies"[36,37]. These include frontotemporal dementia, the patients of which have mutations in the tau gene itself[38,39], suggesting that tau has an important role in the pathogenesis of neurodegene rative diseases, including AD.

A prominent hypothesis regarding the aging process proposes that the accumulation of oxidative damage on macromolecules is the main cause of cellular senescence[40]. Highly reactive oxygen species oxidize lipids, proteins, and DNA, leading to tissue damage and cell death[41]. In addition to its likely role in senescence, oxidative stress has also been implicated in neurodegeneration. The brain appears to be particularly sensitive to oxidative injury, and there is substantial evidence demonstrating the presence of oxidative damage in brain tissue derived from AD[42,43]. Notably, markers for oxidized lipids and proteins accumulate in regions that are particularly affected by neurodegene ration[44,45]. Anti-oxidant constituents of SG[20] may be favorably contributing to the memory improving effect seen in the present study. Thus, the protective effect of SG may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. In the present study, SG when administered for 5 d to young and older mice showed significant reduction of whole brain acetylcholinesterase activity thereby probably facilitating cholinergic transmission and improving memory of animals. SG (50 and 100mg/kg, p.o.) improved the memory of mice as reflected by diminished TL and enhanced SDL values as compared to control animals. Furthermore, animals were protected from memory deficits produced by scopolamine and diazepam. Phenytoin (12mg/kg, p.o.) significantly elevated brain AChE activity. SG was found to be more or equivalent in potency of Piracetam in improving learning abilities and memory capacities of mice. Ethanolic

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and aqueous extract of SG (50 and 100mg/kg, p.o.) for 5d consecutively showed significant reduction in total serum glucose and cholesterol levels of young and aged mice. The animals administered with SG did not show any gross behavioral changes during the experimental period. Thus, SG appears to be a suitable and worthwhile plant that needs to be explored further for its potential in retarding the onset of Alzheimer's disease.

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