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Anti-Alzheimer's disease activity of methanolic extract of *Sesbania Grandiflora* Leaf

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ABSTRACT

Alzheimer's is a disease of the brain that cause problems with memory, thinking and behavior. It is not a normal part of ageing. Alzheimer's disease is a degenerative brain disease and is the most common cause of dementia. Recognized factors in Alzheimer's disease include acetylcholine deficiency, increased free radicals and inflammation of the brain tissue. Drugs designed to slow down the disease progression are available. However these medicines have drawbacks of inducing central and peripheral side effects, insomnia, etc. Thus the demand for the new and safer drugs from natural sources have become important. *Sesbania grandiflora* is an important plant belonging to family Fabaceae which is commonly known as agathi. The reported uses are Anti- convulsant, anxiolytic, Anti-microbial, anti- diarrhoeal and anti-inflammatory. The main active constituents present are alkaloids, flavonoids, tannins and phenolic compounds. Main objective of this study is to establish the anti – Alzheimer's disease activity of methanolic extract of leaves of *Sesbania grandiflora* by using various *in vivo* and *in vitro* methods. *In vitro* determination of Anti-Alzheimer's Disease activity by the neuroprotective effect determination by MTT assay. *In vivo* anti – Alzheimer's activity evaluated by stepdown method. Elevated plus maze model: Albino mice divided in to five groups each of 6 animals as follows. Group I: Normal, Group II: Control, Group III: Standard (Donepezil), Group IV & V: Test (High and low doses). After seven days of drug treatment, animals are allowed to explore the maze for 2 min and transfer latency (TL) is measured on seventh and eighth day.

Keywords: *Sesbania grandiflora*, active constituents, *in vitro* *in vivo* studies

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INTRODUCTION

Alzheimer's disease (AD) is one of the most common neurodegenerative disease and accounts for more than 80% of dementia cases worldwide in elderly people. It leads to the progressive loss of mental, behavioral, functional decline and ability to learn. AD affects more than 40 million people worldwide. The principal risk factor is age: its incidence doubles every 5 years after age 65, and the odds for a diagnosis of AD after age 85 exceed one in three. With the disproportionate growth of the elderly population, the prevalence of AD is predicted to approach around 115 million worldwide in 2050. The total costs for AD in 2013 were approximately US\$205 billion in the USA alone and about US\$605 billion worldwide, not including the contributions of unpaid caregivers. Thus, AD has become a major public health and socio-economic problem that threatens to become the scourge of the 21st century. The clinical and neuropathological diagnosis of AD, as well as the future treatment options, are the focus of the present project.

Neuropathologists have identified amyloid plaques and NFTs in the brains of people with AD, suggesting that these pathologies cause the disease. Amyloid plaques are extracellular deposits of A β in the brain parenchyma and in the cerebral blood vessels where it is known as congophilic angiopathy also known as cerebral amyloid angiopathy (CAA). NFTs composed largely of paired helical filaments with hyperphosphorylated tau proteins, neuronal and synaptic loss.

Rivastigmine, galantamine, tacrine, donepezil, memantine are the currently US FDA approved drug used in the treatment of Alzheimer's disease. AD is associated with inadequate levels of this important neurotransmitter¹. Each drug acts in different way to delay the breakdown of neurotransmitter Ach. However, these drugs fail to completely cure the disease, which warrants a search for newer class of targets that would eventually lead to effective drugs for the treatment of AD. This is pertinent and timely information for researches in this field as existing therapies in the field, which are limited in number, have shown disappointing results. As pointed out by the author's "*Plants provide wealth of bioactive compounds, which exert a substantial strategy for the treatment of neurological disorders such as Alzheimer's disease.*"²

Sesbania grandiflora is native to Asia and is now widespread in most humid tropical regions of the world. It is often cultivated on the low dikes between rice fields or in association with Guinea grass. *Sesbania grandiflora* is a fast-growing perennial, deciduous or evergreen legume tree, up to 10-15 m high. Its lifespan is about 20 years. Its roots are heavily nodulated and some floating roots may develop in waterlogged conditions. The trunk is straight with few branches. The leaves, up to 30 cm long, are pinnately compound with 20-50 oblong leaflets, 1-4 cm long and 0.5-1.5 cm broad. The flowers are white, yellowish, pink or red and borne in axillary racemes. The pods are

50-60 cm long, glabrous and indehiscent, and hang vertically. They contain 15 to 50 dark brown seeds, 5 mm long and 2.5-3 mm broad.

The tanins, flavonoides, coumarins, steroids and triterpens more or less important contents according to the intensity of coloring obtained. The alkaloids are generally found in the form of traces. The saponosides were more often present in methanolic extracts than aqueous extract. The saponosides would be rather present in the form of triterpens and steroids that in the form of heterosides. Leucocyanidin and cyanidin are the active ingredients of *Sesbania grandiflora*. 3-rutinoside are the major chemical constituents of flower. The bark contains tannins and gum. Saponin and Sesbanimide isolated from seeds.^{3,4,5}

All parts of *Sesbania grandiflora*. areutilized for medicine in Southeastern Asiaand India including preparations derivedfrom the roots, bark, gum, leaves, flowers,and fruit. In Folk Medicine it is resorted to be aperient, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic. Agati is a folk remedy for bruises, catarrh, dysentery, eyes,fevers, headaches, smallpox, sores, sorethroat, and stomatitis.

The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect while the flowers have been reported to have antimicrobial activity, hypolipidemic, antiulcer and anti-inflammatory effects.^{6,7}



Figure 1: Leaf of *Sesbania grandiflora*

MATERIALS AND METHOD

Plants Material

The leaves of *Sesbania grandiflora* were obtained from Payyavoor, Kannur, Kerala (India) in the month of November 2018 and authenticated by Dr Abdul Jaleel, Department of Botany, Sir Syed College, Taliparamba, Kannur. A voucher Specimen APSC/COL/06/2018 was deposited in the Department of Pharmacology, Academy of Pharmaceutical Sciences, Gov. Medical College, Kannur, and Kerala.

Extraction

After authentication the plants were collected, cleaned and the leaves were dried in shade at room temperature. The dried leaves were pulverized in a mechanical grinder obtain coarse powder. The powder (250g) was sieved through sieve No. 40 and the powder was subjected for maceration with 500ml of methanol (99.5%) for 72 hrs separately. The extract was then shaken, filtered through muslin cloth and marc was discarded. The filtrate obtained from plant material by maceration was evaporated and stored for further use.

Preliminary Phytochemical Investigation^{4,5,6,7,8}

The methanolic extract of *Sesbania grandiflora* was subjected to phytochemical tests and it was found that the extract contains alkaloids, flavonoids, glycosides, fixed oils, carbohydrates, proteins, amino acids, saponins, tannins and phenolic compounds.

Animals

Healthy Swiss Albino mice (15-25 g) of either sex were selected for the study. The animals were procured from Animal Breeding Home, Govt. Veterinary College, Mannuthy, Thrissur, Kerala. The animals were housed in animal house, Academy of Pharmaceutical Sciences, Pariyaram. The animals were put in polypropylene cages and maintained under a standard environmental conditions; room temperature 25±20C, 12 h dark cycle and 65±10% relative humidity with free access to food (rodent pellet) and water ad libitum. The animals were acclimatized for at least 5 days to then laboratory conditions before doing behavioral experiments. The studies were conducted after obtaining the approval from the Institutional Animal Ethical Committee (CPCSEA/IAEC-18/19-03), Academy of Pharmaceutical Sciences, Pariyaram, Kannur.

In Vitro Neuroprotective Activity Was Determined By MTT Assay⁹.

SH-SY5Y (Neuroblastoma cells) cell line was purchased from National Centre for Cells Sciences (NCCS) Pune, was maintained in Dulbecco's modified eagles media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagle medium (Gibco, Invitrogen).

The cell lines was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µ/ml), and Amphotericin B (2.5µg/ml). Culture cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBSE pendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted Phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10⁴ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

Preparation of Compound stock:

The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation:

After attaining sufficient growth, 150µM Amyloid was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator.

Cytotoxicity Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength reader at a wavelength of 540nm (Laura B. Talarico et al., 2004).

The percentage viability was calculated using the formula:

$$\% \text{ Viability} = \text{Mean optical density of sample} / \text{Mean optical density of control group} \times 100$$

ACUTE TOXICITY STUDIES ¹⁰

Toxicity studies of leaf extract of *Sesbania grandiflora*

Acute toxicity studies of the *Sesbania grandiflora* was carried according to OECD Guideline 423.

The animals were selected and grouped as three animals per group. Animals should be fasted prior to dosing (with mice, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animal should be weighed and the test substance administered. Test substance was administered in a single dose by oral route. A single administration of starting dose of 2000mg/kg body weight p.o. of the extract was administered through three female mice. After the extract has been administered food may be withheld for a further 1-2 hr in mice. Where a dose administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the periods.

They were noted individually after dosing at least once during the first 30 minutes, periodically during the first 4 hr. and daily thereafter for a total of 14 days. The mice is observed for three days to evaluate considerable changes in body weight and other sign of toxicity.

The acute toxicity studies of methanolic extract of *Sesbania grandiflora* was found to be safe and nontoxic. Phytochemical data of the plant *Sesbania grandiflora* revealed the presence of bioactive molecules which are mostly nontoxic in nature hence a limit test was carried out with a dose limit of 2000mg/kg.

***In vivo* pharmacological activities.**

Screening of Anti-Alzheimer's disease activity.

Elevate plus maze model¹¹⁻²³

The Elevated plus maze apparatus consisted of two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 12 cm). The arms extended from the central platform (5 cm x 5 cm) and the maze was elevated to a height of 25cm from the floor. On the first day each mouse was placed at the end of open arm, facing away from central platform. TL was recorded on the first day for all animals (ITL). The mouse was allowed to explore the maze for another 2 min and returned to the home cage (i.e, on the seventh day). Retention of this learned task was examined 24 h after the first test (i.e, on the eighth day). Animals are randomly selected as per the above procedure. Animals in the normal group are trained with the above sessions. In the second group of animals the inducing agent Scopolamine (0.4 mg/kg) was given i.p. and after 45 min of single dose of scopolamine administration TL was measured as above.

Animals in the standard group are treated with Donepezil (5 mg/kg) for seven successive days and on the seventh day after 45 min of single dose of scopolamine (i.p.) administration, record the TL. Similarly, in the fourth and fifth group the animals are treated with low and high dose (200 mg/kg, 400 mg/kg) of plant extract was given orally for seven consecutive days and on the seventh day after single dose of scopolamine was given i.p. and 45 min after the injection TL was measured.

On the seventh day transfer latency (ITL) was noted and on the 8th day retention of learned task was noted. On the 9th day animals are sacrificed and brain is isolated for the further biochemical analysis.

Collection of Blood and Brain Samples

After dosing on 7th day, 9th day the animals were sacrificed, the blood was collected. Then whole brain was carefully removed from the skull quickly and placed in ice cold saline. The blood was centrifuged at 3000 rpm for 15 minutes so as to separate the serum. The serum was used for estimation of total cholesterol and glucose levels. One half were weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% sodium chloride solution. The homogenate was centrifuged at 3000rpm for 10 minutes and the resultant cloudy supernatant liquid was used for estimation of brain acetyl cholinesterase (AChE) activity.

Estimation of Brain AchE activity ²⁴⁻²⁷

The whole brain AchE activity was measured using the Ellman method. 0.5 ml of cloudy supernatant liquid was pipette out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB. From the volumetric flask, 4 ml portions were pipette out into test tubes, into one of the test tube 2 drops of serine solution was added. 1 ml acetyl choline iodide solution was added into both the tubes and incubated for 10 minutes at 30°C. Test tube containing serine was used for zeroing colorimeter. The end point is the formation of yellow color because of 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 spectrophotometer. The resulting yellow color is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, changes in absorbance per min of sample was read at 420 nm.

Estimation of Total Cholesterol Level ²⁸⁻³⁰

CHOD-PAP method was used for the estimation of serum total cholesterol. In this method, the blank sample, standard sample and test sample were pipette into the respective reaction vessels using a micropipette. For the blank sample, 20µl of distilled water and 1000µl of working reagent were mixed. For the standard sample, 20µl of standard cholesterol and 1000µl of working reagent, while for the test sample, 20µl of serum and 1000 µl of working reagent were mixed. These mixtures were incubated for 10 minutes at 37°C. The absorbance was read at 510nm and 630nm (Filter1 and Filter 2) against the blank sample by using Auto analyzer (Erba Mannheim Chem-5 plus V2).

Estimation of Blood Glucose Level

GOD-POD method was used for the estimation of blood glucose using Auto-analyzer. In this method, the blank sample, standard sample and test sample was pipette out into the respective reaction vessels using a micropipette. For the blank sample, 10 μ l of distilled water and 1000 μ l of working reagent were mixed. For the standard sample, 10 μ l of standard glucose and 1000 μ l of working reagent, while the test sample, 10 μ l of serum and 1000 μ l of working reagent were mixed. These mixtures were incubated for 15 minutes at 37 $^{\circ}$ C. The absorbance was read at 510nm and 630nm (Filter 1 and Filter 2) against the blank sample by using Auto-analyzer (Erba Mannheim Chem-5 V2)

Statistical Analysis

The results were analyzed for statistically significance by one-way ANOVA in Graph Pad Prism Software (Version 7.01). P values of < 0.05 were considered as statistically significant.

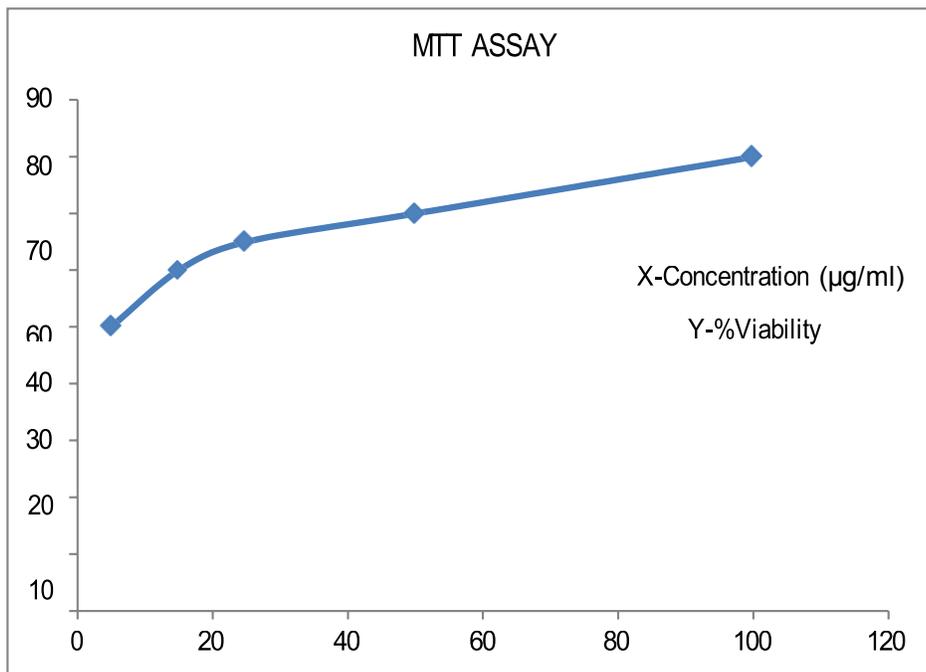
RESULTS AND DISCUSSION

The methanolic extract of *Sesbania grandiflora* was subjected to phytochemical tests and it was found that the extract contains alkaloids, flavonoids, glycosides, fixed oils, carbohydrates, proteins, amino acids, saponins, tannins and phenolic compounds. Root, bark, leaves, sap, flower are commonly used. The leaves extract of plant have reported anti- convulsant, anti- diarrhoeal, anti-inflammatory and immunomodulation activities.

Evaluation of In vitro Neuroprotection in SH-SY5Y cells by MTT assay^{31, 32}

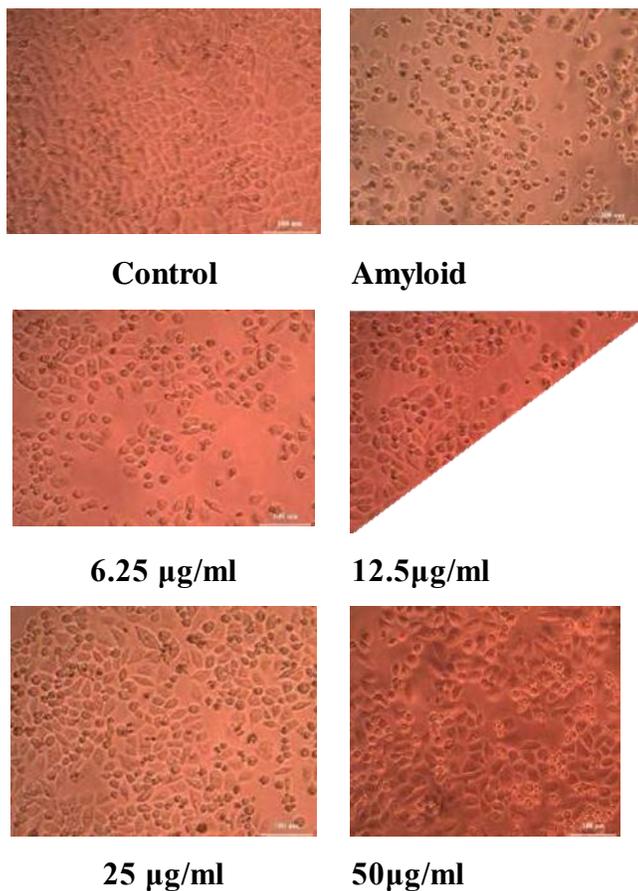
Table 1: Evaluation of neuroprotection by MTT assay

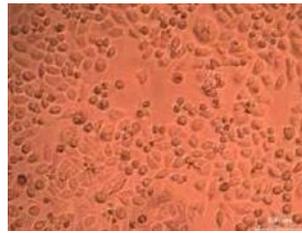
| Groups | Concentration μ g/ml | Average OD at 540nm | Percentage viability |
|---------|-----------------------------|------------------------|-------------------------|
| Control | | 0.5859 | 100 |
| Amyloid | | 0.3039 | 51.59 |
| MESG | 6.25 | 0.3164 | 53.72 |
| | 12.5 | 0.3495 | 59.34 |
| | 25 | 0.3784 | 63.84 |
| | 50 | 0.4172 | 70.27 |
| | 100 | 0.4572 | 77.62 |



% viability of control=100 % viability of amyloid=51.59

Figure 2: *In vitro* neuroprotective activity of *Sesbania grandiflora* by MTT assay





100µg/ml

Figure 3: Cell Viability in *Sesbania grandiflora* by MTT assay at various concentrations

Acute toxicity studies

Acute toxicity were determined in mice by following OECD 423 guidelines. The methanolic extract of *Sesbania grandiflora* were found to be safe up to 2000mg/kg body weight by oral route. After 24 hours, animals were found well tolerated. There was no mortality and sign of toxicity after 14 days. From this the doses for the study of anti- Alzheimer's disease activity was selected as metabolism of drugs in animals is 5 -10 times more than human individuals. So the doses selected for the present study are 200mg/kg and 400mg/kg lower dose and higher dose respectively.

Evaluation of anti-Alzheimer's disease activity

Elevated Plus Maze model

Transfer latency (TL) on seventh and eighth day of drug treatment reflected improvement of both learning and memory. The mice treated with MESHG showed a dose dependent reduction in TL of both seventh and eighth day indicating significant improvement in both learning and memory.

Table 2: Effect of MESHG on Transfer latencies of mice

| Group | Transfer latency on 7th day (sec) | Transfer latency on 8th day (sec) |
|--|-----------------------------------|-----------------------------------|
| Group 1- Normal | 110.1 ± 0.0076**** | 109.33 ± 0.7149**** |
| Group II-Toxic control | 137.01 ± 0.024 | 135.2 ± 0.0076 |
| Group III-Donepezil hydrochloride 5mg/kg | 125.3 ± 0.006**** | 108.1 ± 0.0262**** |
| Group IV-MESHG 200mg/kg | 132.5 ± 0.013** | 115.1 ± 0.0208**** |
| Group V-MESHG 400mg/kg | 128.1 ± 0.22**** | 111.3 ± 0.0076** |

Values are expressed as mean ± SEM of 6 mice in each group, and the values were compared to toxic control. The values were analyzed by one way ANOVA followed by Dunnett's multiple comparison test. **** Significant at P < 0.0001, *** significant at P < 0.001, ** significant at P < 0.01.

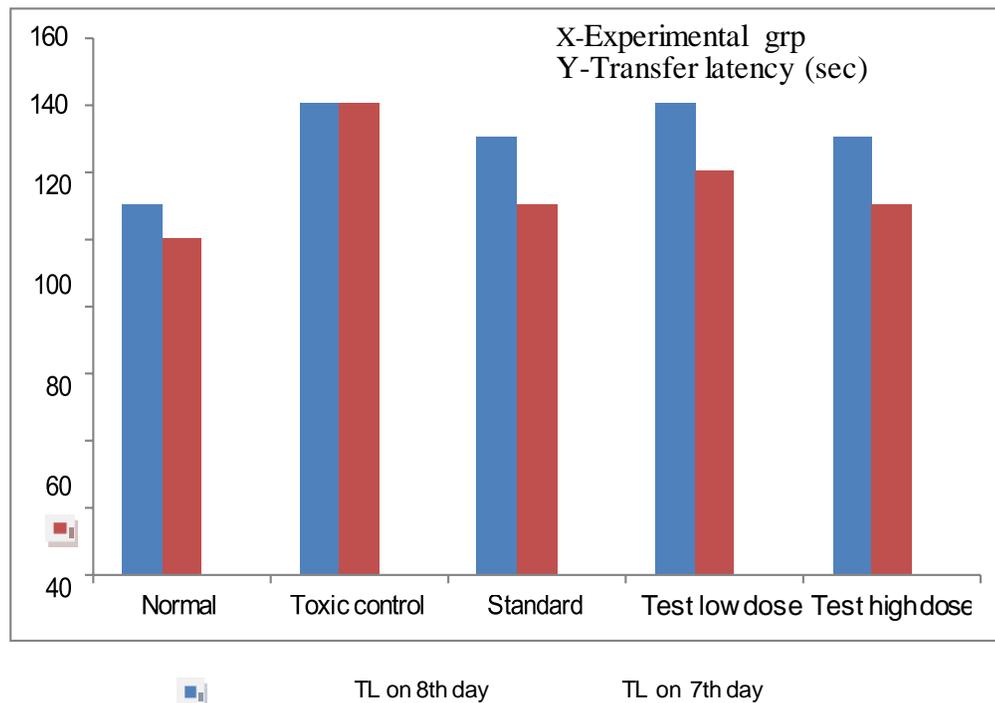


Figure 4: Effect of *Sesbania grandiflora* on transfer latencies in mice

In the present investigation MESG have evaluated for the anti-Alzheimer's activity against scopolamine induced amnesia using evaluated plus maze model. Donepezil was taken as a reference standard in the present study. Donepezil is the leading compound for the treatment of AD in more than 50 countries. As compared with other conventional acetyl cholinesterase inhibitors (AChEIs), donepezil is a In the present investigation MESG have evaluated for the anti-Alzheimer's activity against scopolamine highly selective and reversible piperidine derivative with AChEI activity that exhibits the best pharmacological profile in terms of cognitive improvements. Scopolamine induced amnesia is an interceptive behavioural model is widely used as a primary screening test since scopolamine interferes with memory and cognitive function in humans and experimental animal by blocking muscarinic receptors and produce transient memory deficit.^{33,34} Elevated plus maze model served as the exteroceptive behavioural model to evaluate memory in mice whereas step down method is used to evaluate long term memory in mice.

Transfer Latency (TL) of seventh and eighth day of treatment reflected improvement of both learning and memory. The mice treated with MESG showed dose dependent reduction in TL of both seventh and eighth day indicating significant improvement in both learning and memory i.e., for test lower dose 132.5 ± 0.013 and 115.1 ± 0.020 , and for test higher dose 128.1 ± 0.22 and 111.3 ± 0.0076 . Toxic control increased the TL of seventh day and eighth day indicating impairment in both learning and memory and reversed the amnesia induced by scopolamine. The mice treated with standard drug showed a value 125.3 ± 0.006 and 108.1 ± 0.0208 on seventh and

eighth day. The higher test dose values were almost close to the standard values, this signifies that the test extracts had a comparable effects as the standard drug.

Determination of brain acetyl cholinesterase activity

Table 3: Effect of MESG on brain AChE activity in mice

| Group | AchE level (μ moles/min/mg Protein) |
|--|---|
| Group I-Normal | 0.224 \pm 0.0007*** |
| Group II-Toxic control | 0.7468 \pm 0.0080 |
| Group III- Donepezilhydrochloride 5mg/kg | 0.3242 \pm 0.0012*** |
| Group IV-MESG 200mg/kg | 0.5210 \pm 0.008*** |
| Group V-MESG 400mg/kg | 0.4307 \pm 0.002*** |

Values are expressed as mean \pm SEM of 6 mice in each group, and the values were compared to toxic control. The values were analyzed by one way ANOVA followed by Dunnett's multiple comparison test. **** Significant at $P < 0.0001$, *** significant at $P < 0.001$.

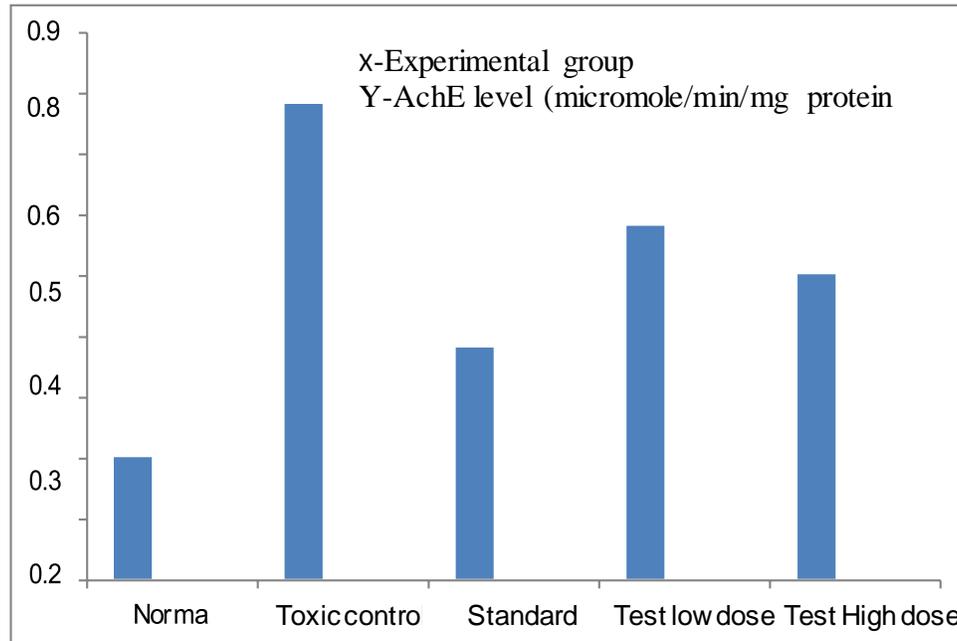


Figure 5: Effect of MESG on AChE activity in mice

On the basis of result obtained from biochemical estimation in MESG (200 mg/kg, 400 mg/kg) treated animals, it can be postulated that *Sesbania grandiflora* significantly reduced brain AChE activity in mice. This suggests that the memory enhancing activity of *Sesbania grandiflora* might due to inhibition of AChE, leading to increase in brain acetylcholine level. From the results the standard drug treated mice had AchE level of $0.324 \pm 0.0012 \mu$ moles/min/mg Protein, and those treated with test extract 200 and 400 mg/kg had 0.521 ± 0.008 and $0.4307 \pm 0.002 \mu$ moles/min/mg Protein respectively. From the results, it was evident that both the doses of test, as the dose increases test extract showed better inactivation of AChE.

BIOCHEMICAL ESTIMATION

Effect on serum cholesterol level

The animals, which received test drug, MESG (200 mg/kg and 400 mg/kg) and standard drug, donepezil hydrochloride (5 mg/kg) orally for 7 days showed significant reduction in total cholesterol level compared to toxic control group which exhibited higher cholesterol level compared to normal control group. The extract thus possesses a promising cholesterol lowering property.

Effect of blood glucose level

Toxic control group showed higher level of glucose in blood compared to normal group. The animals that received test drug, MESG (200 mg/kg and 400 mg/kg) and standard drug, donepezil hydrochloride (5 mg/kg) orally for 7 days showed significant reduction in blood glucose level compared to toxic control group.

Table 4: Effect of MESG on cholesterol and glucose levels of mice

| Group | Glucose(mg/dL) | Cholesterol(mg/dL) |
|--|--------------------|--------------------|
| Group I-Normal | 870.26 ± 0.083**** | 140.4 ± 0.0763**** |
| Group II-Toxic control | 137.2 ± 0.349 | 156.6 ± 0.0160 |
| Group III-Donepezil hydrochloride 5mg/kg | 89.43 ± 0.113**** | 110.4 ± 0.084*** |
| Group IV-MESG 200mg/kg | 120.3 ± 0.0094*** | 138.4 ± 0.763*** |
| Group V-MESG 400mg/kg | 105.3 ± 0.007*** | 125.2 ± 0.0107**** |

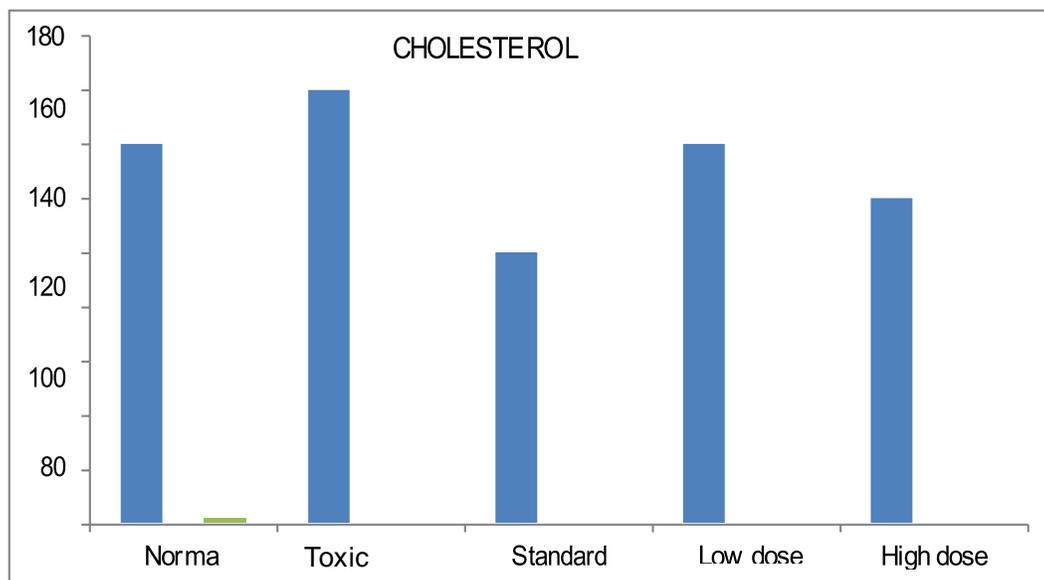


Figure 6: Effect of MESG on cholesterol levels of mice

Values are expressed as mean ± SEM of 6 mice in each group, and the values were compared to toxic control. The values were analyzed by one way ANOVA followed by Dunnett's multiple comparison test. ****Significant at P < 0.0001, *** significant at P < 0.001.

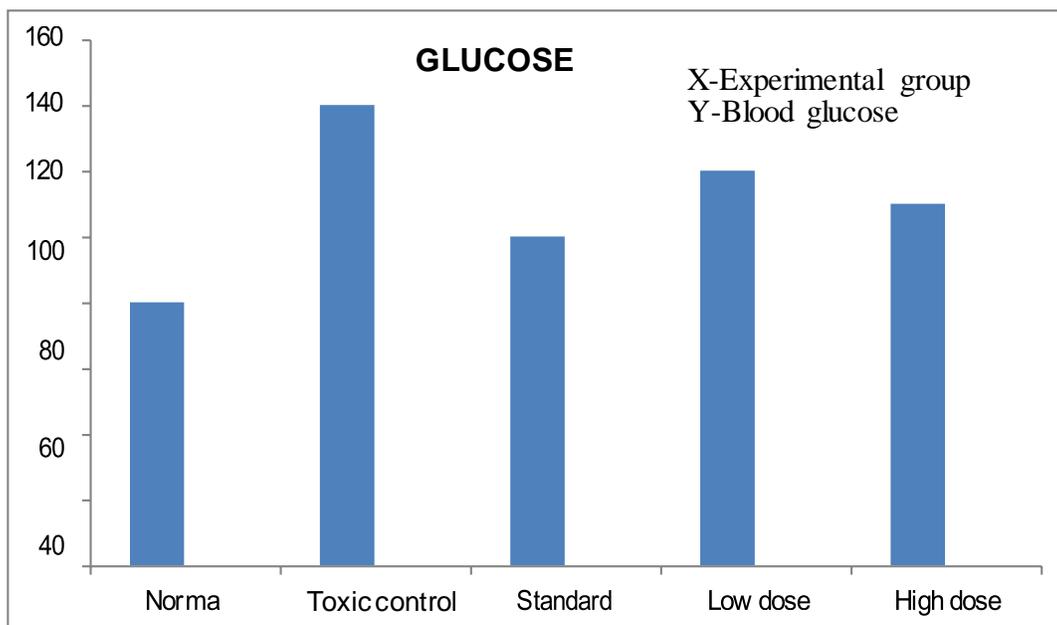


Figure 7: Effect of MESG on glucose levels of mice

The animals, which received test drug, MESG (200 mg/kg and 400 mg/kg) and standard drug, donepezil hydrochloride (5 mg/kg) orally for 7 days showed significant reduction in total cholesterol level compared to toxic control group which exhibited higher cholesterol level compared to normal group. So the methanolic extract has significant cholesterol lowering activity. The level of cholesterol in mice were found to be 138.4 ± 0.0763 mg/dl ($p < 0.001$), 125.2 ± 0.0107 mg/dl ($p < 0.0001$) at doses of MESG 200 and 400 mg/kg respectively, whereas the animals treated with standard drug Donepezil hydrochloride showed cholesterol level as 110.4 ± 0.082 mg/dl ($p < 0.001$). From the results it is evident that high dose of test extract had a comparable effects with that of standard drug.

Toxics control group showed higher level of glucose in blood compared to normal group. The animals that received test drug, MESG (200 mg/kg and 400 mg/kg) and standard drug, donepezil hydrochloride (5 mg/kg) orally for 7 days showed significant reduction in blood glucose level compared to toxic control group. The extract thus possessed a promising glucose lowering property. The level of glucose in mice were found to be 120.3 ± 0.0094 mg/dl ($p < 0.001$), 105.3 ± 0.007 mg/dl ($p < 0.001$) at doses of MESG 200 mg/kg and 400 mg/kg respectively, whereas the animals treated with standard drug donepezil hydrochloride showed glucose level as 89.43 ± 0.113 mg/dl ($p < 0.0001$). High dose of test extract have a comparable effects with that of the standard drug.

DISCUSSION

Sesbania grandiflora. is an important medicinal plant with rejuvenative properties used in Ayurveda for promoting vitality and life. In India, the plant is native to humid, subtropical environments and is quite common in Kerala and Karnataka. In Ayurveda various parts of plant have been used as a remedy for various ailments. Root, bark, leaves, sap, flower are commonly used. The leaves extract of plant have reported anti-convulsant, anti-diarrhoeal, anti-inflammatory and immunomodulation activities.

The methanolic extract of *Sesbania grandiflora* was subjected to phytochemical tests and it was found that the extract contains alkaloids, flavonoids, glycosides, fixed oils, carbohydrates, proteins, amino acids, saponins, tannins and phenolic compounds.

In vitro neuroprotection study of the MESHG was evaluated by MTT assay in SH-SY5Y cell lines. The result indicated that the MESHG possessed good neuroprotective activity.

Acute toxicity studies were already conducted in mice by using OECD guidelines. For MESHG, and it was found that 2000 mg/kg was safe dose. 200 mg/kg and 400 mg/kg doses were selected for testing anti-Alzheimer's disease activity as lower dose and higher dose respectively.

The anti-Alzheimer's disease activity was studied using elevated plus maze model, and it showed dose dependent increase in memory.

After the studies in elevated plus maze, animals were sacrificed, blood and brain samples were collected. Biochemical parameters like cholesterol, glucose levels in blood and AchE in brain homogenate were measured. The serum blood level of cholesterol and glucose were decreased in MESHG treated group compared to the standard confirmed the anti-Alzheimer's disease activity. Reduction of brain AchE activity and protection of brain from neurodegeneration by the extract also confirmed the anti-Alzheimer's disease activity.

CONCLUSION

Sesbania grandiflora is an important medicinal plant widely distributed throughout India. Preliminary phytochemical studies of the leaves extract of *Sesbania grandiflora* have been performed here in this thesis. These studies revealed the presence of alkaloids, carbohydrates, proteins, amino acids, saponins, tannins and phenolic compounds. Further molecular advanced studies can be done so that phytochemical constituents can be specifically identified.

The extract was found to poses neuroprotective, memory enhancing, and acetylcholine esterase inhibitory properties. Methanolic extract of *Sesbania grandiflora* showed significant activity in elevated plus maze at a dose of 200mg/kg and 400mg/kg. Above results were promising and enlighten the scope of further dose studies in the plant. Neuroprotective activity by preventing

neurodegeneration, and anticholinesterase property by facilitating central cholinergic transmission also contributes to the overall activity of *sesbania grandiflora* in central nervous system.

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