MEMORY ENHANCING ACTIVITY OF *Desmodium gangeticum* ROOT EXTRACT ON SCOPOLAMINE INDUCED SWISS ALBINO MICE

A dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

Registration number: **261426065**

Under the guidance of

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CHENNAI– 600003

APRIL 2016

CERTIFICATE

This is to certify that the dissertation entitled "**MEMORY ENHANCING ACTIVITY OF *Desmodium gangeticum* ROOT EXTRACT ON SCOPOLAMINE INDUCED SWISS ALBINO MICE**" submitted by the **Register number: 261426065**in partial fulfilment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmacology** by The Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work done by during the academic year 2015-2016 under the guidance of Mrs. R. Indumathy, M.Pharm., Asst. Professor in Pharmacy, Madras Medical College, Chennai -03.

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CONTENT

ABBREVATIONS

AD	Alzheimer’s disease
AChE	Acetylcholinesterase
Ach	Acetylcholine
DG	*Desmodium gangeticum*
NFTs	Neurofibrillary tangles
CMC	Carboxy methyl cellulose
DTNB	5,5’ –dithio-bis-bis-2-nitrobenzoic acid
O.D	Optical Density
OPT	O-Phthalaldehyde
SD	Standard Deviation
ANOVA	Analysis of variances
B7C	Bis(heptyl)-cognitin
B73	Bis(propyl)-cognitin
NMDA	N- methyl d- aspartate
CPE	*Cissampelospariera*
SOD	superoxide dismutase
CAT	Catalase
GPX	glutathione peroxidase
i.p	Intra peritoneal
p.o	Per oral

1. INTRODUCTION

Memory is one of the most essential roles of the brain. Memory is vital for survival because it is the process by which organisms are able to record their experiences and use this information to adapt their responses to the environment. Loss of memory and cognitive impaired functions are the major features of Alzheimer's disease (AD). Presence of acetylcholine within the neo cortex is sufficient to ameliorate learning deficits and restore memory. Decreased cholinergic firing in brain, rise in oxidative stress, hypercholesterolemia, and neuroinflammatory reactions have been demonstrated to play an etiological role in memory decline. The central cholinergic system is involved in cognitive functions and plays an important role in learning and memory for humans and animals[1].

Alzheimer's disease (AD) is progressive irreversible neurodegenerative disorder that was first identified and written by Dr. Alois Alzheimer in early 1900s. It occurs gradually and results in cognitive impairment, unusual behaviour, personality changes, an ultimately death. It is the most common form of adult onset dementia. Presently, it is the 4th leading cause of death in western countries, preceded only by heart disease, cancer and stroke.

The world Alzheimer report 2015 led by king's college London found that there are currently around 46.8 million people living with dementia around the world, with numbers projected to nearly double every 20 years, increasing to 74.7 million by 2030 and 131.5 million by 2050.**In India 4.1 million of people are living with dementia**. There are an estimated 5.3 million Americans of all ages with Alzheimer's disease. In 2015, an estimated 7,00.000 Americans age ≥65 years will die with AD, and many of them will die from complications caused by AD[2].

The term "dementia" describes a loss of mental ability associated with gradual death of brain cells. The most common cause of dementia in the elderly is probably Alzheimer's

disease (AD), a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language[3].

Amnesia is the general term for a condition in which memory (either stored memories or the process of committing something to memory) is disturbed or lost, to a greater extent than simple everyday forgetting or absent mindedness.

Amnesia may result either from organic or neurological causes (damage to the brain through physical injury, neurological disease or the use of certain drugs), or from functional or psychogenic causes (psychological factors, such as mental disorder, post-traumatic stress or psychological defence mechanisms)[4].

CAUSES OF ALZHEIMER'S DISEASE[4]

It is a neurodegenerative disease, which means there is progressive brain cell death that happens over a course of time. The total brain size shrinks with Alzheimer's – the tissue has progressively fewer nerve cells and connections. In Alzheimer's disease, brain cells start to deteriorate. The body attempts to stop this process by producing a protein called amyloid. However amyloid deposits build up in the brain, leading to further deterioration.

These deposits of amyloid are referred to as "plaques" and cause the brain cells to shrivel up and form "tangles", which in turn lead to changes in the brain structure and cause the brain cell to die.

The formation of plaques and tangles also prevents the production of some important brain chemicals, called neurotransmitters. Over time the loss of brain cells cause the brain to shrink.

Figure: 1

PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE[6-8]

The pathophysiology of Alzheimer's disease is complex, involving many neurotransmitter systems and pathophysiologic processes.

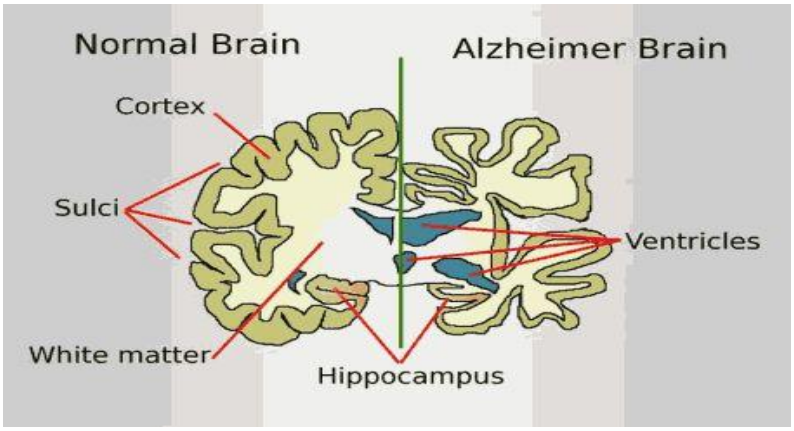
- ✓ Change in brain structure
- ✓ Degenerative processes in Alzheimer's disease
  - ➢ β amyloid hypothesis
  - ➢ The cholinergic hypothesis
  - ➢ The glutamatergic/ excitotoxicity hypothesis
  - ➢ The oxidative stress hypothesis
  - ➢ The chronic inflammation hypothesis

3. Other neurotransmitter deficiencies
4. Cholesterol

1. Changes in brain structure

In this disease, brain atrophy and neuronal loss occur in a predictable pattern beginning with the entorhinal cortex and hippocampus, ultimately progressing throughout the brain.

Figure: 2



2. Degenerative processes in Alzheimer's disease[6-8]

This disease is characterized by 3 neuropathologic hallmarks:

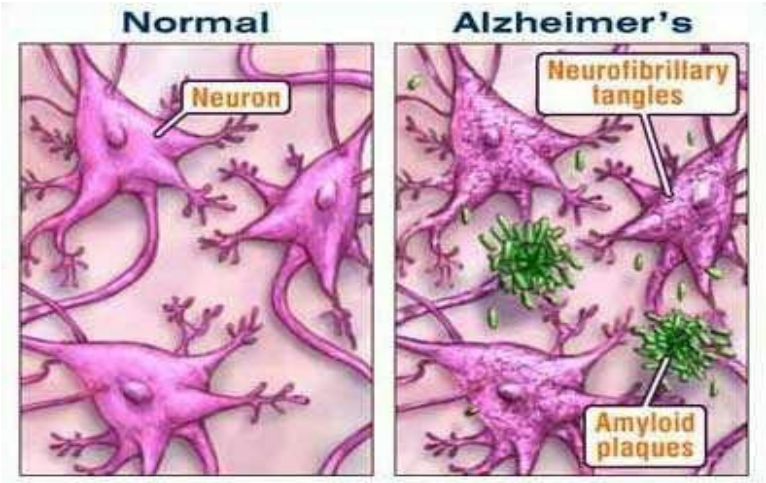
- ✓ Plaques of β-amyloid protein
- ✓ NFTs
- ✓ Neuronal degeneration

All of which occur during normal aging, but in disease occur in specific location in the brain.

β-amyloid plaques

These are thought to play the central role in AD pathogenesis, described as the "amyloid cascade"

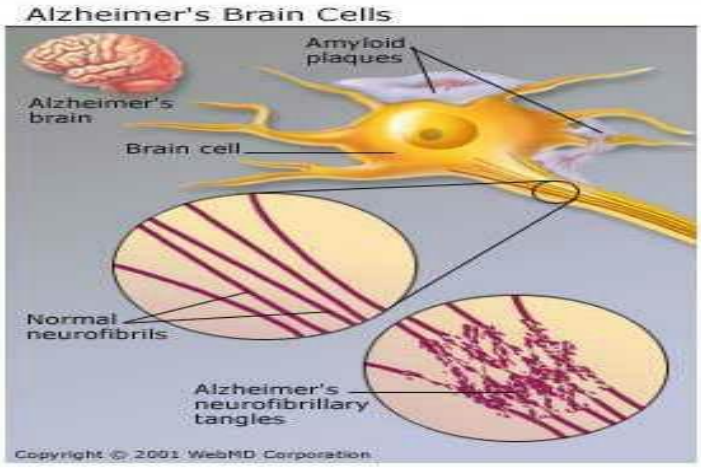
Figure: 3



Neuro Fibrillary Tangles

The β-amyloid hypothesis maintains that formation of β-amyloid plaques is the first step in AD pathology and genetic and histopathologic studies support this hypothesis. NFTs occur after plaque formation.

Figure: 4



The cholinergic hypothesis

This is supported by the drastic and widespread loss of cholinergic neurons in AD, the benefits with acetylcholinesterase inhibitors and the role of the nicotinic cholinergic receptor.

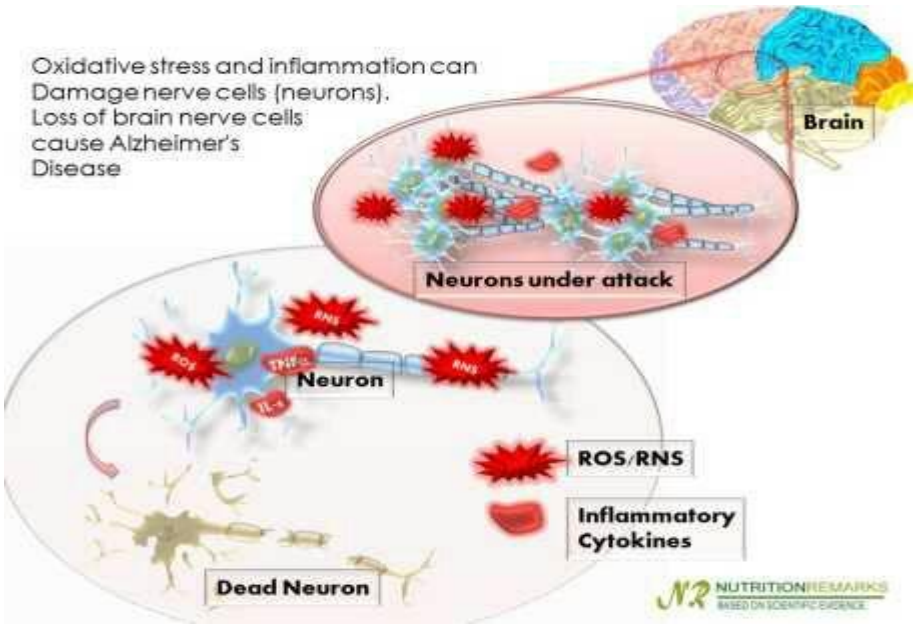
The glutamate/excitotoxicity hypothesis

It maintains that slow but steady activation of a glutamate receptor leads to cellular damage.

The oxidative stress hypothesis

It suggests that β- amyloid induces oxidative stress causing permanent damage to some neuronal macromolecules and creating reactive species, which further propagate neuronal toxicity.

Figure: 5



The chronic inflammation hypothesis

This inflammation observed with Alzheimer's disease is thought to be the result of all of these mechanisms causing cell damage and death. In Alzheimer's disease, inflammatory cytokines respond to these processes and contribute to neuronal destruction because they appear to act uninhibited[6, 7].

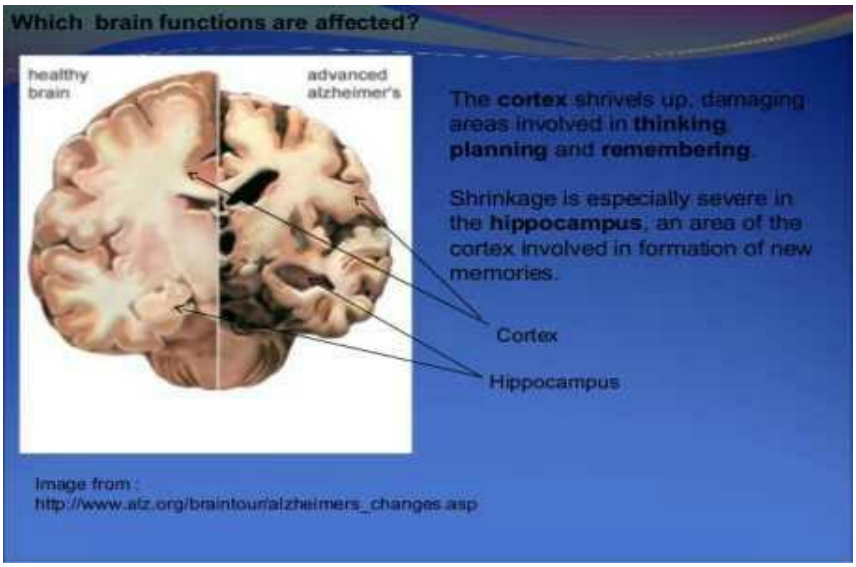
Other neurotransmitters deficiencies

Serotonin and norepinephrine are also involved in Alzheimer's disease pathology. Serotonin is an important factor in depression and anxiety, both of which are common in patients with Alzheimer's disease.

Cholesterol

This is also appears to have a role in Alzheimer's disease progression, perhaps through a reduction in vascular risk factors, which are common in patients with Alzheimer's disease

Figure: 6



STAGES OF ALZHEIMER'S DISEASE[8-10]

STAGE ONE: FORGETFUL

Mild or early stages, lasts for 2 to 8 years. Person needs occasional reminders, lists and routine.

- ✓ Short term memory loss.
- ✓ Disorientation to time; spatial disorientation.
- ✓ Aphasia, mild anomia, some circumlocution.
- ✓ Mild apraxia.
- ✓ Judgment errors.
- ✓ Affect changes.
- ✓ Absent-minded, difficulty concentrating.
- ✓ Behavior or lifestyle changes.
- ✓ Self-aware or unaware of deficits or changes.

STAGE TWO: CONFUSION

Middle/Moderate stage, lasts for 3 to 6+ years. Person needs occasional assistance and supervision.

- ✓ Short-term and remote memory loss.
- ✓ Needs help with activities of daily living (bathing, toileting, etc.)
- ✓ Emotional ups and downs.
- ✓ Aphasia, paraphasia with semantic/phonemic errors.
- ✓ Ideational and ideomotor apraxia. One or two steps skills.
- ✓ Agnosia; less able to interpret sensory input (visual, touch, etc.)
- ✓ Restless, listless, wandering or slow moving, hard to motivate (abulia).
- ✓ Clumsy movement, decreased muscle control and some ataxia.

STAGE THREE: DEMENTIA

Severe or end stage lasts for 1 to 4 years. Person needs constant supervision and assistance. Respite essential to primary caregiver.

- ✓ Decreased communication skills, difficulty talking or understanding.
- ✓ Apraxia, one step skills.
- ✓ Perseveration.
- ✓ Minimal motor control.
- ✓ Forgets social graces, decreased gating, and spontaneous outbursts.
- ✓ Behavioral problems, such as wandering, unwilling to bathe or dress.
- ✓ Incontinent.
- ✓ Bedridden.

Identified risk factors for developing the condition include:

- ✓ Increasing age
- ✓ History of the head injury
- ✓ Down's syndrome
- ✓ Risk factors for blood vessels disease such as smoking
- ✓ Family history of Alzheimer's disease
- ✓ Obesity
- ✓ High blood pressure
- ✓ High cholesterol
- ✓ Insulin resistance.
- ✓ Genetic factor
- ✓ Environmental factor

SIGNS AND SYMPTOMS OF ALZHEIMER'S DISEASE [13, 62, 64]

Memory loss

Forgetting recently learned information is one of the most common early signs of dementia.

Difficulty performing familiar tasks

People with dementia often find it hard to plan or complete everyday tasks.

Problems with language

People with this disease often forget simple words or substitute unusual words, making their speech or writing hard to understand.

Disorientation to time and place

People with this disease can become lost their neighbor's, forget where they are and how they got there, and not know how to get back home.

Poor or decreased judgment

Those with Alzheimer's disease may dress inappropriately, wearing several layers on a warm day or little clothing in the winter. They may show poor judgment.

Problems with abstract thinking

Someone with Alzheimer's disease may have unusual difficulty performing complex mental tasks, like forgetting what numbers are and how they should be used.

Misplacing things

A person with Alzheimer's disease may put things in unusual places, an iron in the washing machine or a watch in the freezer.

Change in mood or behavior

Someone with Alzheimer's disease may show rapid mood swings- from calm to tears to anger- for no apparent reason.

Change in personality

The personalities of people with dementia can change dramatically. They may become extremely confused, fearful or dependent on a family member.

Loss of initiative

A person with Alzheimer's disease may become very passive, sitting in front of the television for hours, sleeping more than usual or not wanting to do usual activities

TREATMENT OF ALZHEIMER'S DISEASE[14-19]

Treatment of Alzheimer's disease there is no cure for Alzheimer's disease and drug therapy for the disease is still in its infancy.

Acetylcholinesterase inhibitors help improve memory function and attention in Alzheimer's disease patients by interfering with the breakdown of acetylcholine, thereby increasing the levels of the neurotransmitter at the synapse. There are currently three FDA-approved cholinesterase inhibitors: rivastigmine and galantamine(for mild to moderate Alzheimer's disease), and donepezil (for all stages of Alzheimer's disease)[15].

Memantineis another FDA-approved medication for use in moderate to severe Alzheimer's disease but belongs to a different class of drugs known as NMDA (glutamate) receptor antagonists.

Antioxidants such as vitamin-E (α tocopherol) monoamino oxidase inhibitor (selegiline), phenolics (curcumin), tannins (gallic acid) and polyphenolics (ferulic acid) reduce the free radical formation and prevent the cognitive syndromes.

Several vaccines are under development to reduce the cognitive symptoms due to Alzheimer's disease.

The discovery of novel Alzheimer's diseasetreatments and has been used in the past for other neurodegenerative disorders (e.g., anti-viral drug amantadine for use in Parkinson's disease)[15,16].

Nootropic agents such as aniracetam, piracetam,and pramiracetam and choline esterase inhibitors like donepezil, rivastigmine are being primarily used to improve memory, mood and behaviour.

According to the WHO more than 80% of the world's population relies on traditional herbal medicine for their primary healthcare. In recent time there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs.

As we know that India, with its mega biodiversity and knowledge-rich ancient traditional system of medicine viz Ayurveda, Siddha, Unani and local health traditions,provides a strong base for the utilization of a large number of plants in general healthcare of the people[5].

*Desmodium gangeticum*and reported that the alkaloids indole -3-alkylamines and β carbolines has biological activities like anticholinesterase, smooth muscle stimulant and CNS stimulant response. And also studied the chemical composition of the roots and reported three pterocarpeniods, **gangetin, gangetinin**and **desmodin** respectively[5].

Gangetin, a pterocarpan, shows anti-fertility activity by affecting alkaline phosphatase activity in uterine fluids. It is reported to possess antiulcer, antioxidant, cardio tonic, anti-inflammatory, anti-nociceptive activities and useful in neurological disorders. Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various

cognitive disorders. **Gangetin,gangetinin**and **desmodin**are the major chemical constituents present in the root of *Desmodium gangeticum*[5].

About 38 different species of Desmodium have been reported in India of which *Desmodium gangeticum*and *Desmodium adscendens* are used ethno medically all over the world.Among which *Desmodium gangeticum* is used in the Indian system of medicine; particularly in the Ayurveda is an important and well explored species of genus Desmodium. *Desmodium gangeticum* contains majorly alkaloids, flavonoids and pterocarpinoids.

The Ayurvedic herbs **Brahmi, Ashwagandha, Curcumin, Arcoruscalamus, Shankhpushpi, Zingiber**, etc help to improve Alzheimer's situation[17, 18].

Ultimately, the most successful model of treatment for Alzheimer's disease will likely include early detection and control of physical factors (diabetes, hypertension, hyperlipidemia), followed by application of multifaceted, disease-modifying interventions to prevent the early and continued loss of neurons and to reduce the toxins that result in further cell deterioration[15].

This plant is one among the Dashamoola (roots) of Ayurveda and is an important ingredient of many famous Ayurvedic drugs like **Dashamoolarishta(**anemia, haemorrhoids, liverdisease**), Chyavanaprasha(**it act against the ageing process, anti-oxidant**), Dhanwantharam Kuzhambu(** useful in treatment of neurological conditions such as Neuritis, Neuralgia**), Rasnadhi decoction(**Rheumatoid arthritis**), Agusthya Rasayanam(**Respiratory disease**), Sukumbaragritham(**used an inflamations**), DasamulaKatuthiayadikashayam(**anti-inflammatory**), Dasamulathailam(**ascites**), Danvantrathailam(**headache, Neuro muscular conditions**), Mahamasahthailam(**Widely used for many neurological conditions**), Anuthailam(**improve memory and functions of four

sense organs and also nervous disorders**), Vidaryadigritham(**used in treatment for myalgia**)** and **Brahma Rasayan(**it helps to fight tiredness, fatigue, stress and aging**)**[5]**.**

This study was undertaken to evaluate the potential benefits of *Desmodium gangeticum* (fabaceae) root using mice as animal model for the treatment of Alzheimer's disease.

2. AIM AND OBJECTIVE

- *Desmodium gangeticum* has been reported to possess antioxidant activity and anti-inflammatory activities are known to be associated with Nootropic activity.
- In this plant, the aerial part of *Desmodium gangeticum* has been shown to possess anti-amnesic activity. No work has so far been carried out on the roots of *Desmodium gangeticum*.

Hence, the aim and objective of the present study is to,

- To extract the root of *Desmodium gangeticum* by hot continuous extraction using Soxhlet apparatus using various solvents n-hexane, ethyl acetate and ethanol.
- To evaluate the neurotransmitter (acetylcholinesterase and serotonin) estimation of ethyl acetate and ethanol root extracts of *Desmodium gangeticum* by *ex vivo* method.
- To evaluate the memory activity of ethyl acetate and ethanol root extracts of *Desmodium gangeticum* by *in vivo* (Morris water maze and Y maze) method.

3. REVIEW OF LITERATURE

- ✓ **Vaghela *et al.***(2013) was demonstrated the *Desmodium gangeticum* is quite promising as a multipurpose medicinal agent. So further clinical trials should be performed to prove its efficacy[20].

- ✓ **T. ShriVijayaKirubha *et al.*** (2011) was demonstrated the *Desmodium gangeticum* serves as one of the main ingredient of famous Ayurvedic preparations. Thus, the utility of *Desmodium gangeticum* as a medical plant has increased many folds over a period of time[21].

- ✓ **Prashar*et al.***(2014) was demonstrated that protocatechuic acid has potential therapeutic effects on improving the anti-amnesic activity in rats through inhibiting lipid peroxidation and decreasing acetylcholinesterase (AChE) in brain[23].

- ✓ **Yi-fan Han *et al.***(2012) was concluded that B3C and B7C might provide greater therapeutic efficacy for the treatment of AD by concurrently acting on multiple targets's, including inhibiting AChE, blocking NMDA receptor and antagonizing $GABA_A$ receptor[24].

- ✓ **Kulkarni *et al.***(2011) was investigated the effect of *Cissampelos pariera* on learning and memory in scopolamine induced mice. Based on results of experiments, found that CPE had remarkable cognitive enhancing activity[25].

- ✓ **Mehrdad jahanshahi*et al.*** (2011) was concluded that scopolamine, as a non-selective muscarinic receptor antagonist, can significantly shorten the latency

compared to the saline control group in test day. Also it can reduce the number of neurons in sub-regions of hippocampal formation[26].

- ✓ **Ajay J Parikh *et al***.(2013) was concluded guggul extract is a hypolipidemic agent and can be exploited as antialzheimer's agent and also shows significant synergistic effect when co-administered with piracetam[27].

Anti-inflammatory activity

- ✓ **R. Govindarajan *et al.***(2007) has been studied the flavonoid and alkaloid fractions of *Desmodium gangeticum* were evaluated for anti-inflammatory activities in carrageenan-induced inflamed rats with the aim of studying the promising fraction for inhibitory action on ferrous sulphate induced lipid peroxidation, superoxide dismutase (SOD), catalase(CAT), glutathione peroxidase (GPX) and total reduced glutathione in liver and spleen homogenates of inflamed rats[22].

- ✓ **AnshuRathi*et al.*** (2004) has been studied the water decoction of root and aerial parts of *Desmodium gangeticum* was examined for anti-inflammatory activity in experimental animals. There was a significant increase in analgesio-meter-induced force equivalent to6.56–67.66% protection and 22.18–73.83% protection in acetic acid-induced writhing[20].

- ✓ **D. Ghosh and A. Anandakumar** (1983) were reported the Gangetin isolated from the hexane extract of the root of the plant *Desmodium gangeticum*. The compound showed significant anti-inflammatory activity in the exudative and the proliferative phases of inflammation in the doses of 50 and 100mg/kg orally in albino rats[69].

- ✓ **ShivaniGhildiya *et al.***(2013) was studied that *Laghupanchamula* denotes combinations of roots of five herbs. However, in Ayurvedic classics besides four common herbs viz. Kantakari, Brihati, Shaliparni and Prinshniparni, the fifth one is either Gokshura (Laghupanchamula with Gokshura LPG) or Eranda (Laghupanchamula with Eranda LPE), and both formulations have been documented to have shothahara (anti-inflammatory) action. Compare the anti-inflammatory activity of 50% ethanolic extract of LPG and LPE were studied in in rats and in mice. LPG and LPE significantly reduced acute and sub-acute inflammation and showed effective and similar anti-inflammatory activity[70].

Anti-nociceptive activity [68]

- ✓ **AnshuRathi *et al.***(2004) was studied the water decoction of root and aerial parts of *Desmodium gangeticum* was examined for anti-nociceptive activity in experimental animals. The result establishes the traditional use of water decoction of *Desmodium gangeticum* codified in Indian System of Medicine.

Analgesic activities[20]

- ✓ **D. Ghosh and A. Anandakumar**(1983) were reported the Gangetin isolated from the hexane extract of the root of the plant *Desmodium gangeticum*. The compound showed significant analgesic activity in albino rats.

Anti-amnesic activities[19]

- ✓ **Joshi Hanumanthachar and Parle Milind** (2007) has been studied the effectiveness of aqueous extract of *Desmodium gangeticum* (whole plant) in attenuating scopolamine-induced amnesia in mice. It increased mice brain acetylcholine content and decreased acetyl cholinesterase activity in a similar manner to the standard cerebro-protective drug piracetam. Hence, aqueous extract of D. gangeticum can be

used to delay the onset and reduce the severity of the symptoms of dementia and Alzheimer's disease. .

Anti-oxidant activity

- ✓ **R. Govindarajan *et al.*** (2006) has been studied the total alcoholic extract of *Desmodium gangeticum*, which exhibited significant anti-inflammatory activity and was evaluated for the possible mode of action by studying its antioxidant potential in adjuvant-induced arthritic rats[28].
- ✓ **Prakash Veeru1 *et al.*** (2009) was studied some medicinal plant extracts for anti-oxidant activity and after ending of study he was consulted the methanolic crude extracts of *Desmodium gangeticum* found to be the strongest antioxidant, followed in descending order by *Amaranthuscaudatus, Solanumnigrum, Piper longum, Eclipta alba* and *Ocimum sanctum*[20].
- ✓ **Gino A Kurian *et al.*** (2009) was reported the effect of aqueous extract of *Desmodium gangeticum* root in different antioxidant models and experimentally induced ischemic reperfusion in an isolated rat heart. The rats were divided into three groups namely control, reperfusion control and drug treated. The above results suggest that the aqueous extract of DG root exhibit potential free radical scavenging effect that can reduce the oxidative stress exhibited by IRI[29].
- ✓ **Jen-Chieh Tsai *et al.*** (2009) was studied antioxidant activities and phenolic components of the crude extracts of 10 Desmodium species from Taiwan. In this study, DPPH free radical scavenging activity, ABTS radical monocation scavenging activity, ferric-reducing antioxidant power (FRAP) and reducing power of the 10 Desmodium species were evaluated for their antioxidant activities[20].

Anti-pyretic activity

- ✓ **Zhan-Zhou Zhu *et al.*** (2006) was studied the petroleum ether fraction (PEF) from the ethanol extract of *Desmodium gangeticum*, which finding significantly antipyretic activities in mice[20].

Anti-diabetic activity[20]

- ✓ **Govindarajan R. *et al.*** (2007) was studied the insulin secretion and anti-diabetic activity of *Desmodium gangeticum*. Treatment of diabetic rats with aerial parts of *D. gangeticum* extract (DG, 100 and 250mg/kg body weight) for 3 weeks showed a significant reduction in blood glucose. *D. gangeticum* extract caused a significant increase in insulin secretion from MIN6 cells grown as monolayers and as pseu-doislets, indicating that the antidiabetic activity may be as a result of increased insulin secretion. It also had a role on the lipid profile of the rats by causing reductions in cholesterol and triglycerides and increasing the HDL significantly.

Cardiovascular activity

- ✓ **G. A. Kurian *et al.***(2008) was evaluated the effect of a methanol extract of *Desmodium gangeticum* (L) DC (Fabaceae) (DG) on lipid per-oxidation and antioxidants in mitochondria and tissue homogenates of normal, ischemic and ischemia-reperfused rats. The results of their study showed that DG possesses the ability to scavenge the free radicals generated during ischemia and ischemia reperfusion and thereby preserves the mitochondrial respiratory enzymes that eventually lead to cardio protection[30].

- ✓ **G. A. Kurian and Jose Paddikkala** (2012) was studied mimetic action of herbal extract *Desmodium gangeticum* (DG) roots on ischemia reperfusion injury. The results with physiological parameters like left ventricular developed pressure, end diastolic pressure and working index of isolated rat heart showed significant recovery in DG root extract administrated rat heart. So they concluded DG methanol root extract provides myocardial protection towards IRI by stimulating muscarinic receptors[29].

- ✓ **M. M. Shabi and L. Upadhyaya**(2012) was studied the effect of *Desmodium gangeticum* on lysosomal hydrolases, phosphatases and electrolytes in mechanically induced myocardial ischemic injury in rats. They concluded that alteration in this enzyme activities may lead change in the electrolytes such as sodium, potassium and calcium content in the heart during ischemic reperfusion injury[20].

- ✓ **Sankar *et al***. (2013) was studied Cardiac hypertrophy occurs in response to increased workload, such as hypertension or valvular heart disease. Oxidative stress has been implicated in cardiac hypertrophy and in its transition to heart failure. The methanolic root extract was analyzed for total phenolic content and tested for antioxidant potential. The results demonstrated potent free radical scavenging activity of DG. Cell line studies showed significant increase in ROS generation, and permeability transition pore opening in ISO-treated cells. This study is the first documentation of the modulatory effect of DG on cardiac hypertrophy[20].

Anti-ulcer activity

- ✓ **Dharmani P *et al***. (2005) was reported anti-ulcerogenic property of ethanolic extract of *Desmodium gangeticum* against cold restraint (CRU, 2 hr cold restraint stress),

aspirin (ASP, 150 mg/kg orally), alcohol (AL, absolute alcohol 1 ml/200gm) and pyloric ligation (PL, 4 hr pylorus ligation) induced gastric ulcer models in Sprague Dawley rats and histamine (HST, 0.25 mg/kg) induced duodenal ulcer in guinea pigs. Treatment with DG showed increase in mucin secretion by 56.17%, whereas OMZ showed 12.45% increase. Anti-ulcer effect of DG may be due to its cytoprotective effect along with antisecretory activity and could act as a potent therapeutic agent against peptic ulcer disease[33].

- ✓ **Ayyavu Mahesh *et al*.** (2005) has been studied the ethanolic root extract of *Desmodium gangeticum* in various acute and chronic ulcer mouse models. Oral administration of root extract, significantly decrease the ulcer index and lesion number in a dose dependent manner against ethanol induced acute gastric ulcer in mice. In gastric ulcerated animal that received high dose of 150mg/kg, the mucosa showed no ulceration with slight focal congestion and the glands appeared normal. The highest dose (150mg/kg) of the extract provoked a marked increase in protein and glutathione levels, when compared to control. Our results indicate that the DG possess gastroprotective activity and increasing regeneration of damaged gastric mucosa and thus safe for human use[20].

CNS activity

Alzheimer's disease[34]

- ✓ **M Obulesu and D. MuralidharaRao** (2011) were studied the extracts of plants and their effect on the amelioration of AD symptoms has been extensively. The mechanisms like acetylcholinesterase (AChE) inhibition, modification of

monoamines, antiamyloid aggregation effect and antioxidant activity which are actively entailed in the process of amelioration of AD symptoms.

- ✓ .**Jabbar S *et al.*** (2001) was studied aqueous extract of *Desmodium gangeticum* and showed severe anti-writhing activity in the acetic acid-induced abdominal writhing assay. It exhibited moderate central nervous system depressant activity in the spontaneous motoractivity, hole cross and open field tests and hole board tests. The effect of this extract on locomotion was compared with some standard CNS drugs.

Antibacterial activity[20]

- ✓ **Krishnasamy Karthikeyan *et al.*** (2001) antibacterial activity of *D. gangeticum* was tested with various solvents viz., methanol, ethanol, chloroform and aqueous extract against various bacterial pathogens such as klebsiellapneumoniae, escherichia coli, salmonella typhi, streptococcus mutants and pseudomonas aeruginosa. Antibiotic sensitivity assay was performed with amoxicillin, kanamycin, tetracycline, ciprofloxacin and penicillin. The result of the selected several extract, the methanolic extract showed maximum zone of inhibition against S. mutants and minimum zone of inhibition was observed with aqueous extract against P. aeruginosa. In addition the antibiotic sensitivity was observed with kanamycin, tetracycline, and ciprofloxacin against all bacteria's. The methonalic extract of D. gangeticum be able to use as potential antibacterial source for various infective pathogens.

Wound Healing Activity

- ✓ **V. Jain, V. Prasad and R.S. Pandey** (2006) were evaluated the aqueous extract of *Desmodium gangeticum* for its wound healing potential on different experimental models of wounds in rats. The aqueous extract of aerial part of DG, in powdered form was incorporated in ointment (10% w/w dried powder in simple ointment base) and was evaluated for wound healing potential in an excision, incision and dead space wound model in rats. The DG ointment showed significant responses in all three-wound types tested when compared with the control group. The effect produced by the DG ointment, in terms of wound contracting ability, wound closure time, tensile strength of the wound, regeneration of tissues at wound site were comparable to those of a standard drug povidone iodine ointment[20].

PLANT PROFILE

BOTANICAL NAME : *Desmodium gangeticum*

FAMILY : Fabaceae

SYNONYM : Prisniparni,Prthkparni, Chitraparnyahi, Kalasi, Dhavaniguha,Simhapucchi, ParnyapiKrestuvinna.

REGIONAL NAME [20, 21]

Sanskrit	:	Shalparni
Tamil	:	Pulladi, Orilai
Telugu	:	Gitanaram, Kolakuporna
Bengal	:	Salpani, Shalpani, Chhalani.
English	:	Flax weed, Flix weed.
Gujarati	:	Salvan,Shalvan,Sameravo,Pandadiyo.
Hindi	:	Shalpan,Saivan,Sarivan,Gauri,Sar,Salpani,Dinth.
Kannada	:	Kadanga,Maru,Nabiyalabune,Nariyalavona,Bhui,Shevara.
Konkani	:	Salvan
Malayalam	:	Orila,Pullati.
Marathi	:	Salvan,Ranbhal.
Mundari	:	Oterai

TAXONOMIC CLASSIFICATION

Kingdom Plantae - Plants

Subkingdom Tracheobionta - Vascular plants

Superdivision Spermatophyta - Seed plants

Division Magnoliophyta - Flowering plants

Class Mangnoliopsida - Dicotyledons

Subclass Rosidae

Order Fabales

Family Fabaceae - pea family

Genus – Desmodium Desv. - ticktrefoil

Species - *Desmodium gangeticum* (L.) DC.

Part used : Roots

Figure: 8

Figure: 9

HABITAT[20, 21]

The plant of *Desmodium gangeticum* is bitter, sweet, thermogenic, nervetonic, febrifuge, digestive, anticatarrhal, antiemetic, aphrodisiac, demulcent, anthelmentic, cardictonic, anti-inflammatory, diuretic, hemostatic, rejuvenating and useful in neuromuscular and ophthalmic disorders, loss of appetite, flatulence, diarrhoea, dysentery, piles, helminthiasis[21].

The root of *Desmodium gangeticum* is one of the constituent of famous Ayurvedic preparation Dasmoola kvatha which is antipyretic and bitter tonic. It is reported to be beneficial in treatment of typhoid fever, biliousness and also diuretic and aphrodisiac.

DISTRIBUTION

Common Species on lower Hills and Plains throughout India ascending to 1500m in the Himalayas. It is frequently found in outer Himalaya, Punjab, Tirunelveli, Kerala in forest and west lend of Bihar and Orissa, Palghat in Madhya Pradesh in open and wasteland forests of Rajasthan forest of Ganjam to Godavari, Ghats from South Canara to Travancore[21].

DESCRIPTION

- ✓ *Desmodium gangeticum* which is commonly known as Saivan(Guajarati),Shalpan(Hindi) and Flax weed (English).It is a stout herb or under shrub, up to 1m.high,
- ✓ Stem is angled, more or less hairy.
- ✓ Leaves are 1-fololate,stipules is scarious, up to 8mm. long.
- ✓ Leaflets-membranous,7-15*3-7cm,ovate – oblong or broadly ovate, acute or acuminate, rounded at the base.

- ✓ Flowers is in large terminal and axillary racemes, usually in small fascicles on a slender rachis; bracts subulate, up to 4mm, calyx-2mm long,hairy,
- ✓ Corolla 4-5mm.long. purple, violet, blue, lilac, or white, these colours appearing at the same time on the same plant
- ✓ Pods are slightly falcate, up to 18mm.long; joints 6-8, longer then broad, slightly hairy with minute hooked hairs[20,21].

CHEMICAL CONSTITUENTS

PLANT

N,N-dimethyitryptamine, 5-methoxy-N,N-dimethyltryptamine, and their Nb-oxides, Nbmethyltetrahydroharman, 6-methoxy-2-methyl-β-carboliniumderivative, Nbmethailtetrahydroharman, hypaphorine, hordenine, caudicine, N-methyltyramine, β phenylethylamine.

ROOT

Three pterocarpenoides**, gangetinin, gangetin** and **desmodin** are the major chemical constituents of the roots.

ETHANOMEDICAL USE

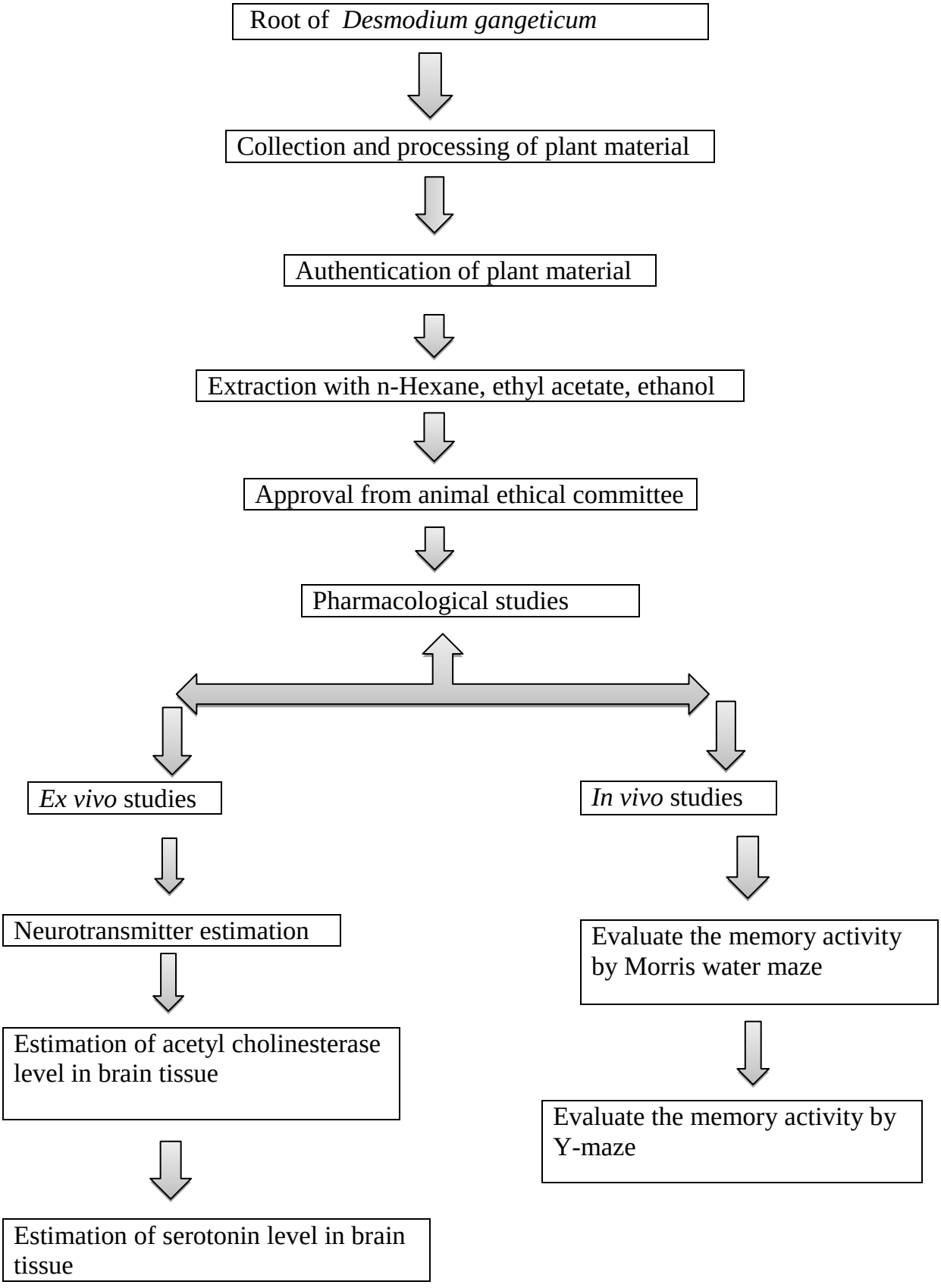
The plant of *Desmodium gangeticum* is bitter, sweet, thermo genic, nervine tonic, aphrodisiac, demulcent, anthelmintic, cardiac tonic, febrifuge, anti-inflammatory, diuretic, hemostatic, rejuvenating, and useful in neuromuscular and ophthalmic disorders, loss of appetite, flatulence, diarrhea, dysentery, nausea, piles, helminthiasis.It is used in angina pain, cardiac disorders, tuberculosis, cough, seminal weakness, urinary disorders, fever, debility and gout.

The root of *Desmodium gangeticum* is one of the constituent of famous Ayurvedic preparation Dasmoola kvatha which is antipyretic and bitter tonic.It isreported to be

beneficial in treatment of typhoid fever, biliousness and also diuretic and aphrodisiac. The root is nervine tonic, diuretic, cardio tonic, and expectorant. The root decoction is used for the treatment of heart diseases, especially in angina pectoris and myocardial infarction. It strengthens heart muscles and reduces cholesterol. Roots are chewed daily for the cure of typhoid and pneumoni[20-21].

The present study was undertaken to assess the memory enhancing potential of *Desmodium gangeticum* root extract in mice.

4. PLAN OF WORK



5. MATERIALS AND METHODS

5.1COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The roots of *Desmodium gangeticum* were collected from Institute of Siddha College, Tirunelveli District, Tamil Nadu (India), in the month of November 2015.The plant material was identified and authenticated by Prof. V. Chelladurai, Research officer- Botany (Scientist-c) (Retd), Central Council for Research in Ayurveda& Siddha, Govt. of India

5.2 PREPARATION OF VARIOUS EXTRACTS OF ROOTS OF *Desmodium gangeticum*

Roots of *D.gangeticum* were washed with distilled water to remove the dirt and soil and shade dried. The dried material were powdered and passed through a 10-mesh sieve. About 50g of air dried powdered plant materials from the roots were transferred into thimble for packing and successing extracted using Soxhlet apparatus by hot continuous percolation method using solvents in the order n-Hexane followed by ethyl acetate and finally ethanol.

A piece of porcelain was added into the round bottom flask to avoid bumping effect. The extracts were filtered using whatmann filter paper No.40 and the solvent were recovered from the extracts under reduced pressure using rotary evaporator.

Table1. The percentage yield from the plant *Desmodium gangeticum* using different solvents

Extract	Weight of plant material used for extraction	Yield (gm)	PercentageYield(%)
n-hexane	**50gm**	**2.0**	**4**
Ethyl acetate	**50gm**	**5.7**	**11.4**
Ethanol	**50gm**	**8.3**	**16.6**

5.3 MEMORY ENHANCING ACTIVITY

5.3.1 Acute toxicity study

Acute toxicity studies have been already done in previous studies and it was found to be safe up to 2000mg/kg.

The selection of *Desmodium gangeticum* dose was based on initial pilot study, whereas scopolamine[19] and piracetam[23] dose selection was based on previous literatures. The memory enhancing activity was investigated on various extracts of DG in Swiss albino mice fed with commercial pellet diet.

5.3.2 EXPERIMENTAL DESIGN

The Institutional Animal Ethical Committee clearance were obtained vide reference **09/243/CPCSEA** dated for the study of memory enhancing activity in ethyl acetate and ethanol extracts of the roots of *Desmodium gangeticum*.

Albino Mice (25-30gm) were procured from Animal House, Madras Medical College, Chennai, Tamil Nadu, India. They were acclimatized for laboratory condition for 7 days and randomly divided into five groups each having six animals. The animals were housed under standard laboratory conditions and maintained under a 12-h light- dark cycle and had free access to drinking water and diet for one week. All the protocols in this study were approved by the ethics committee of madras medical college.

For the pharmacological tests, the obtained extract was suspended in double distilled water containing carboxy methylcellulose (1%w/v CMC) in doses of ethyl acetate and ethanol extract 200mg/kg p.o. The doses were fixed based on earlier studies on the ethyl acetate and ethanol extract of *Desmodium gangeticum* roots extract were administered at up to end of the observation period.

The *Desmodium gangeticum* root extract caused no abnormality or death during the course of treatment.

5.2.3DRUG AND CHEMICALS

Scopolamine was purchased from Sigma. Piracetam was purchased from local medical shop.

5.3.4GROUPING OF ANIMALS[27]

Total 30 animals (Swiss albino mice) were randomly divided into five different groups, each group containing 6 mice and treated for 18 days as follows; Scopolamine(0.4mg/kg)was administered at last three days.

Table: 2

S.No	Group	Name of drug	Dose	No of animals	Duration of dosage
1	Group 1	Control	NIL	6	18days
2	Group 2	Scopolamine	0.4mg/kg	6	Last 3days
3	Group 3	Piracetam+ Scopolamine	200mg/kg	6	18days
4	Group 4	DG ethyl acetate extract+Scopolamine	200mg/kg	6	18days
5	Group 5	DG ethanol extract + Scopolamine	200mg/kg	6	18days

Group 1

It represented the control group.Vehicle was administered orally for eighteen successive days**.**

Group 2

CMC was administered orally for fifteen successive days followed by Scopolamine (0.4mg/kg i.p) after 45 min of administration daily from 16^{th} day to 18^{th} day.

Group 3

Piracetam injection was administered i.p for fifteen successive days followed by Scopolamine (0.4mg/kg i.p) after 45 min of administration daily from 16^{th} day to 18^{th} day.

Group 4

DG ethyl acetate extract was administered orally for fifteen successive days followed by Scopolamine (0.4mg/kg i.p) after 45 min of administration daily from 16^{th} day to 18^{th} day.

Group 5

DG ethanol extract was administered orally for fifteen successive days followed by Scopolamine (0.4mg/kg i.p) after 45 min of administration daily from 16^{th} day to 18^{th} day.

5.3.5 EXPERIMENTAL PROCEDURE

Induction of Amnesia

Amnesia mainly induced by using Scopolamine into the mice. Scopolamine, also known as L-Duboisine, and Hyoscine, is an alkaloid drug with muscarinic antagonist effects. It is among the secondary metabolites of plants from Solanaceae (nightshade) family of plants.

Scopolamine exerts its effects by acting as a competitive antagonist at muscarinic acetylcholine receptors, specifically M1 receptors. Scopolamine is used as a standard/reference drug for inducing amnesia in man and Animals. The effects are generally interpreted as a cholinergic deficit and related to the hypothesis that acetylcholine is involved in memory functions. Scopolamine, besides influencing learning and memory, affects various types of behaviour (e.g., loco motor activity, anxiety, attention).

5.3.6 EXTEROCEPTIVE BEHAVIORAL MODELS

- ✓ Morris water maze
- ✓ Y-maze task

5.3.7 NEUROTRANSMITTER ESTIMATIONS

- ✓ Acetylcholinesterase
- ✓ Serotonin

5.3.6 MORRIS WATER MAZE[24,39]

The procedure used was a modification (Han *et al*., 2009) method described by Morris (1984).

Mice (25-30g) were trained to find a submerged platform (**6.5cmdiameter1cm** below surface) in a circular pool (**diameter60cm; height30cm**) filled with turbidity water (**depth19.5cm; 25±1°C**).

External visual cues were placed around the pool to facilitate navigation of the animals.

The platform was fixed at SW (southwest) direction. Start positions alternated between NE (northeast), SE (southeast), and NW (northwest) direction in a pseudo-random fashion.

Each mouse was placed in the water facing the wall of the pool and allowed to swim for 60s to reach the platform.

Finding the platform was defined as being able to stay on it for at least 2s; mice that crossed the platform without stopping (jumping immediately back into the water) were left to swim until the termination of the trail.

If mouse failed to find the platform in the allotted time, the mouse was manually placed onto the platform manually and assigned as a latency of 60s.

All mice were allowed to rest there for 5mts and then performed the next trial immediately. After the last trail, mice were dried with a cloth and then returned to their home cage.

Mice performed four consecutive trials per day over a 4-day training period. The time to reach the target (escape latency) for each mouse was recorded manually. On the last day (day-5) a probe trail was adopted.

Each mouse started at NE and had to swim freely for 60 s. memory retention was measured by quantifying the time to reach in the target platform.

5.3.6 SPONTANEOUS ALTERATION BEHAVIOR IN THE Y-MAZE TEST[41-43]

We examined continuous spontaneous alternation behaviour using the Y- maze apparatus. The Y-maze apparatus was made of black plastic with three arms (36cm×7cm×13cm) extending from a central platform at 120^0.

Each animal was placed at the end of one arm and allowed to move freely through the Y maze during a session lasting for 8min. Arm entry was defined as the entry of 4 paws into one arm.The sequence of arm entries was recorded visually. Alternation was defined as multiple entries into the 3 arm (A,B or C) on overlapping triplet sets.

The percentage of spontaneous alternation was calculated as the ratio of the actual-to-possible alternations (defined as the total number of arm entries minus 2), multiplied by 100: as shown in the following equation:

Alternation (%) = [(number of alternation)/(total arm entries-2)] ×100

Figure: 10

5.3.7NEUROTRANSMITTER ESTIMATION PROCEDURE[42]

(i) Estimation of Acetylcholinesterase (AChE) Ellman's method

Acetylcholinesterase (AChE) is an enzyme participating in cholinergic neurotransmission. It breaks down acetylcholine which terminates the neurotransmission process. The most common assay is based on Ellman's method using an alternative substrate acetylthiocholine and 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB).

The reaction results in production of 5-thio-2-nitrobenzoate that has yellow color due to the shift of electrons to the sulfur atom.

The animals were sacrificed, whole brains was removed quickly and placed in ice-cold saline. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). 4ml aliquot of the homogenate is added to a cuvette containing 2.6ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly and absorbance was measured at 412nm in a spectrophotometer. When absorbance reaches a stable value, it was

recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance is recorded. Change in the absorbance per minute was determined. Protein estimation was done using folin's method. AChE activity was calculated using the following formula:

Calculations

The enzyme activity is calculated using the following formula

$$\text{Acetylcholinesterase activity (M/ml) } R = \frac{\delta O.D \times \text{Volume of Assay (5ml)}}{E \times \text{mg of protein}}$$

Where,

R= rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed / min/ mg E= Extinction coefficient13600 M-1cm-1

δO.D = change in absorbance

The final reading of enzyme activity is expressed as **µ moles/minute/mg** tissue.

(ii)Estimation of Serotonin[73]

Animals were sacrificed, whole brain was dissected out and the tissues were weighed and were homogenized in5ml HCl–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot of supernatant phase (1ml) was removed and added to centrifuge tube containing 2.5ml heptane and 0.31ml of 0.1MHCl. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two

phases and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken for serotonin assay.

To 0.2ml aqueous extract, 0.25ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectroflurimeter.

5.4 STATISTICAL ANALYSIS

All the values were expressed as mean ±SD. The data was statistically analyzed by one way ANOVA. One way analysis of variance (ANOVA) was used to correlate the statistical difference between the variables. $P< 0.05$, $P<0.001$ was considered to be significant.

6. RESULTS

6.1 *In vivo* methods for memory enhancing activity.

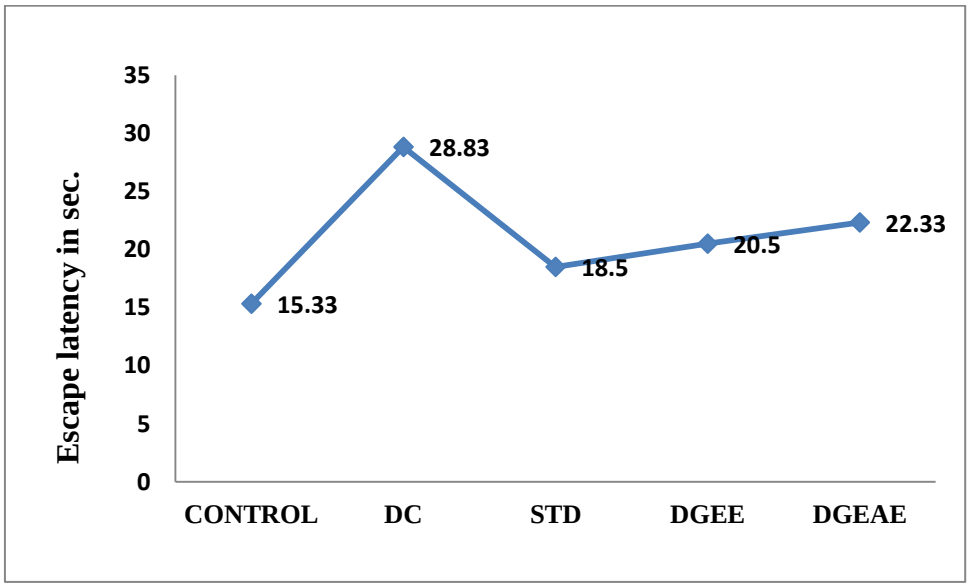
Effect of *Desmodium gangeticum* root extract (ethyl acetate & ethanol) and piracetam on scopolamine induced amnesia in mice using Morris water maze. (Escape Latency Time in Seconds)

Table no 3: Treatment effect on Escape latency in 18th day(By Morris Water Maze)

Group	Treatment	Escape latency
1	Control	**15.33±1.2**
2	Scopolamine(0.4mg/kg)	**28.83±2.1***
3	Piracetam(200mg/kg)	**18.50±0.95****
4	DGEE(200mg/kg)	**20.50±1.2****
5	DGEAE(200mg/kg)	**22.33±1.8****

Values are mean ± SD. (n=6).
***indicates P< 0.001 compared to control,**
**** indicates P<0.001 compared to disease control.**

Figure: 11Treatment effect on Escape latency in 18th day (By Morris Water Maze



The memory enhancing effects of *Desmodium gangeticum* are presented in **table 3**. It was observed that when scopolamine administered, it has significantly (P<0.001) increased escape lantency value (**28.83±2.1**) as compared to the normal group (**15.33±1.2**). When the piracetam was administered for fifteen days at the dose of 200mg/kg, it has significantly (P<0.001) decreased escape lantency value (**18.50±0.95**) as compared to the scopolamine treated group. It was observed that administration of *Desmodium gangeticum* ethanol & ethyl acetate extract at the dose of 200mg/kg resulted in a significant decreased escape latency value(for ethanol **20.5±1.2**, for ethyl acetate **22.33±1.8**) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

There is an increase in escape latency in negative control group when compared with the control group (P<0.001) of the two groups of amnesia induced animals, both showed decreased time to escape on to the escape platform. The group treated with ethyl acetate extract of DG 200mg/kg & ethanol extract of DG 200 mg/kg showed the decreased escape latency and the significance value of (P<0.001) respectively as shown in **table 3.**

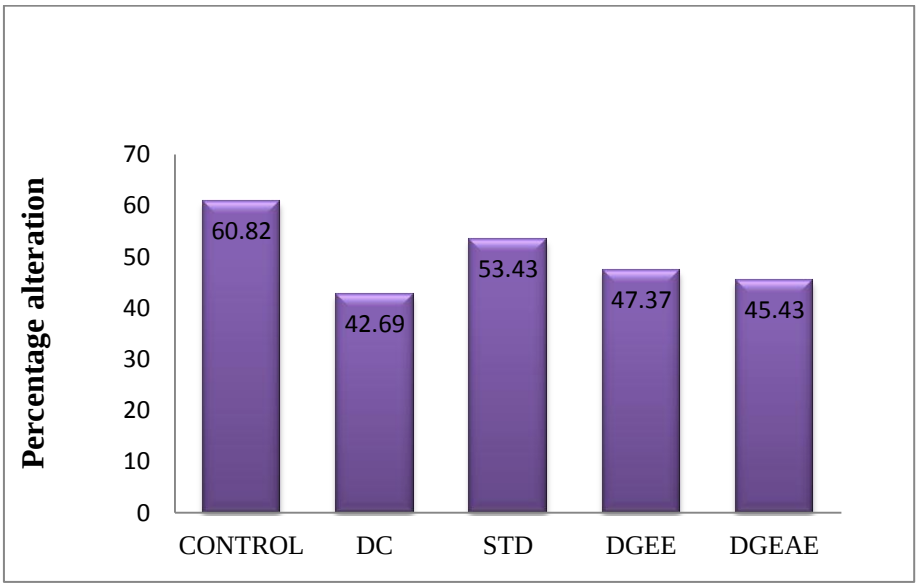
Effect of *Desmodium gangeticum* root extract (ethyl acetate & ethanol) and piracetam on scopolamine induced amnesia in mice using Y- maze.(Percentage alteration)

Table No: 4 Treatment effect on Percentage alteration in 18th day (By Y- Maze)

Group	Treatment	Percentage alteration
1	Control	**60.82±2.6**
2	Scopolamine(0.4mg/kg)	**42.69±3.3***
3	Piracetam(200mg/kg)	**50.39±2.1***
4	DGEE(200mg/kg)	**47.37±6.3****
5	DGEAE(200mg/kg)	**45.43±2.5****

Values are mean ±SD. (n=6).
*** indicates P< 0.001,**
**** indicates P<0.01.**

Figure: 12 Treatment effect on Percentage alteration in 18th day (By Y- Maze)



It was observed that administration of scopolamine resulted in a significant (P< 0.001) decreased percentage alteration value (**42.69±3.3**) as compared to the normal group (**60.82±2.6).** Administration of piracetam for fifteen days at the dose of 200mg/kg, has resulted in significant increased percentage altertion value (**50.39±2.1)** as compared to the scopolamine group. It was observed that administration of *Desmodium gangeticum* ethanol & ethyl acetate extract at the dose of 200mg/kg resulted in a significant (P< 0.01) increased percentage alteration value (for ethanol **47.37±6.3**, for ethyl acetate **45.43±2.5**) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

The amnesia induced group (negative control) indicated decrease in the alternation of behaviour. The results presented by the treatment groups was showed significance by (P<0.01) increase in alteration of behaviour in respect of 200mg/kg of DGEE and 200mg/kg of DGEAE when compared with that of the disease control group. The significance of (P<0.001) (P<0.01) respectively as shown in **table.4.**

6.2 *Ex vivo* methods for memory enhancing activity

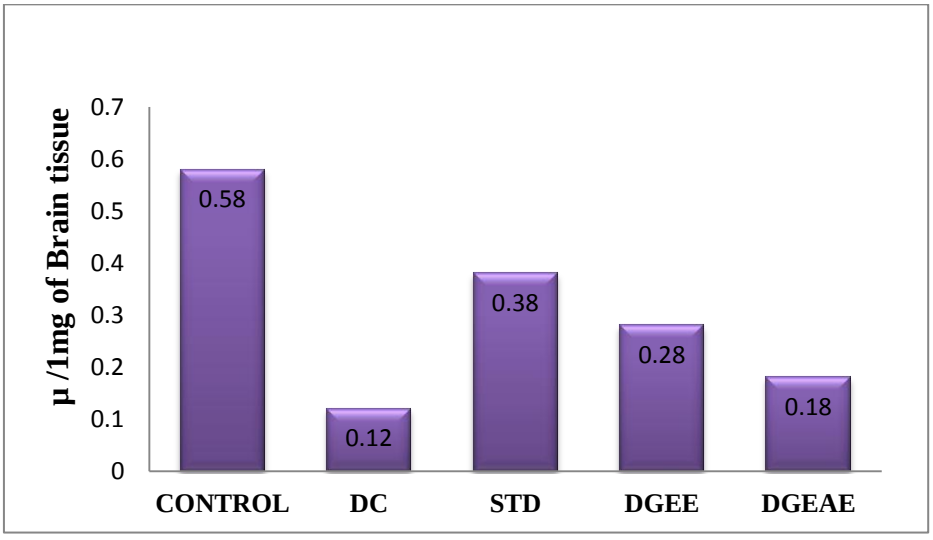
Effect of *Desmodium gangeticum* root extract (ethyl acetate & ethanol) and piracetam on scopolamine induced amnesia in mice using estimation of serotonin activity in µg/1mg of brain tissue.

Table no: 5 Treatment effect on Serotonin activity in brain tissue

Group	Treatment	Serotonin activity (µg/1mg of brain tissue)
1	Control	**0.58±0.01**
2	Scopolamine(0.4mg/kg)	**0.12±0.01***
3	Piracetam(200mg/kg)	**0.38±0.01****
4	DGEE(200mg/kg)	**0.18±0.01****
5	DGEAE(200mg/kg)	**0.28±0.01****

Values are mean ±SD (n=6).
*** indicates P< 0.001 compared to control,**
**** indicates P<0.001 compared to disease control.**

Figure: 13 Treatment effect on Serotonin activity in brain tissue



In this study to determined the level of serotonin in the whole brain homogenate of all group animals, which was used to assess the nootropic activity. It was observed that administration of Scopolamine resulted in a significantly ($P < 0.001$) decreased serotonin values (**0.12±0.01**) as compared to the normal group (**0.58±0.01**). When the Piracetam was administered at the dose of 200mg/kg, it has significantly increased serotonin value (**0.38±0.01**) as compared to the Scopolamine treated group. The activity of serotonin after administration of *Desmodium gangeticum* ethanol & ethyl acetate extract at the dose of 200mg/kg resulted in a significant increased serotonin value (for ethanol **0.18±0.01,** for ethyl acetate **0.28±0.01**) as compared to the Scopolamine treated group. Piracetam, *Desmodium gangeticum* ethanol and ethyl acetate extract has significant nootropic activity as that of Piracetam on scopolamine induced amnesia in mice and also showed synergistic effect.

There is a decrease in serotonin activity in disease control group when compared with the control group ($P<0.001$) of the two groups of amnesia induced animals, both showed decreased in serotonin activity. The group treated with 200&200 mg/kg DGEE & DGEAE showed increased serotonin activity compared with disease control group. The significance of ($P<0.001$) respectively as shown in **table.5.**

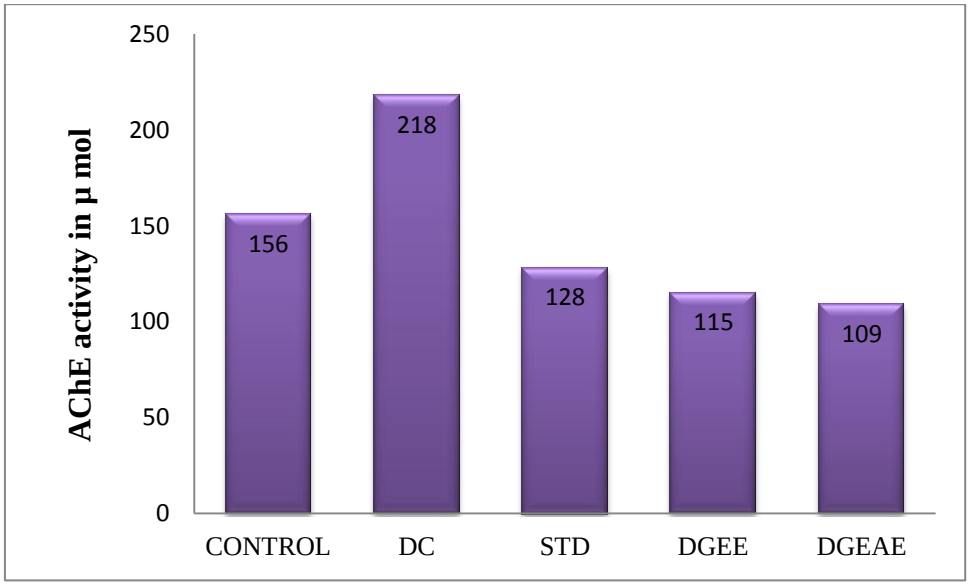
Effect of *Desmodium gangeticum* root extract (ethyl acetate & ethanol) and piracetam on scopolamine induced amnesia in mice using the estimation of AChE activity in µ mol.

Table no: 6 Treatment effect on Acetylcholinesterase activity in µ moles

Group	Treatment	Acetylcholinesterase activity (µmoles)
1	Control	**150±3.39**
2	Scopolamine(0.4mg/kg)	**218±4.19***
3	Piracetam(200mg/kg)	**128±0.77****
4	DGEE(200mg/kg)	**115±1.12****
5	DGEAE(200mg/kg)	**109±1.15****

Values are mean ±SD (n=6).
*** indicates P< 0.001 compared to control,**
**** indicates P< 0.001 compared to disease control..**

Figure: 14Treatment effect on Acetylcholinesterase activity in µ moles



In this study to determined the level of AChE in the whole brain homogenate of all group animals, which was used to assess the nootropic activity. It was observed that administration of Scopolamine resulted in a significantly ($P < 0.001$) increased AChE values (**218±4.19**) as compared to the normal group (**150±3.39**). When the Piracetam was administered at the dose of 200mg/kg, it has significantly decreased AChE value (**128±0.77**) as compared to the Scopolamine treated group. The activity of AChE after administration of *Desmodium gangeticum* ethanol & ethyl acetate extract at the dose of 200mg/kg resulted in a significant decreased AChE value (for ethanol **115±1.12,** for ethyl acetate **109±1.15**) as compared to the Scopolamine treated group. Piracetam, *Desmodium gangeticum* ethanol and ethyl acetate extract has significant nootropic activity as that of Piracetam on scopolamine induced amnesia in mice and also showed synergistic effect.

There is an increase in AChE activity in disease control group when compared with the control group ($P<0.001$) of the two groups of amnesia induced animals, both showed decreased in AChE activity. The group treated with 200&200 mg/kg DGEE & DGEAE showed decreased AChE activity compared with disease control group. The significance of ($P<0.001$) respectively as shown in **table.6.**

7. DISCUSSION

Alzheimer's disease is a genetically heterogeneous neurodegenerative disorder, which is slow in onset but relentless in progress. It is characterized by aphasia, apraxia, and agnosia with the loss of memory as the mine symptoms. Despite the severity and high prevalence of this disease, allopathic system of medicines is yet to provide a satisfactory antidote. Therefore we were motivated to explore the potential of medicinal plants to manage this deadly disease (AD) [4].

Desmodium gangeticum roots were extracted with various solvents like n-hexane, ethyl acetate and ethanol based on its polarity by continuous hot percolation method. The ethyl acetate and ethanol solvents given more percentage of yields compare to n- hexane. So ethanol and ethyl acetate extracts only chosen for *in vivo* and *ex vivo* methods.

In the present study, *D. gangeticum* extract administered orally for 18 days improved the memory of mice as reflected by diminished escape latency and percentage alteration values as compared to control animals. Additionally, *D. gangeticum* reduced central cholinesterase activity, and enhances serotonin activity. Furthermore, pretreatment with *D. gangeticum* for 15 days protected the animals from memory deficits produced by scopolamine. These findings suggested the possible neuroprotective role for *D. gangeticum.*

There is an increase in escape latency in negative control group when compared with the control group ($P<0.001$) of the two groups of amnesia induced animals, both showed decreased time to escape on to the escape platform.

The group treated with ethyl acetate extract of *D. gangeticum* 200mg/kg & ethanol extract of *D. gangeticum* 200 mg/kg showed the significance of (P<0.001) respectively as shown in **table 3.**

The amnesia induced group (negative control) indicated decrease in the alternation of behavior. The results presented by the treatment groups was showed significance by (p<0.01) increase inalteration of behavior in respect of 200mg/kg of DGEE and 200mg/kg of DGEAE when compared with that of the disease control group. The significance of (P<0.001) (P**<**0.01) respectively as shown in **table.4**

There is a decrease in serotonin activity in disease control group when compared with the control group (P<0.001) of the two groups of amnesia induced animals, both showed decreased in serotonin activity. The group treated with 200&200 mg/kg DGEE & DGEAE showed increased serotonin activity compared with disease control group. The significance of (P<0.001) respectively as shown in **table.5**

There is an increase in AChE activity in disease control group when compared with the control group (P<0.001) of the two groups of amnesia induced animals, both showed decreased in AChE activity. The group treated with 200&200 mg/kg DGEE & DGEAE showed decreased AChE activity compared with disease control group. The significance of (P<0.001) respectively as shown in **table.6.**

Nootropic represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performances, learning capability and memory. Piracetam, the first representation of a class of Nootropic agents, has been shown to improve memory deficits in individuals. Repeated injection of piracetam had improved learning abilities and memory capacities of

laboratory animals. Both piracetam and *D. gangeticum* meet major criteria for Nootropic activity, improvement of memory in absence of cognitive deficit[20].

Epidemiological studies have almost confirmed that non-steroidal anti-inflammatory drugs reduce the incidence of Alzheimer's disease. *D. gangeticum* has been shown to produce anti-inflammatory action in rodents. This anti-inflammatory effect of *D. gangeticum* would certainly help Alzheimer's disease patients. Oxygen free-radicals are implicated in the process of age-related decline in cognitive performance and may be responsible for the development of Alzheimer's disease in elderly persons.

D. gangeticum has been reported to possess antioxidant property as well. The neuroprotective effect of *D. gangeticum* may attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduce brain damage and improved neuronal functions[22].

The symptoms of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the affected brain areas. Cognitive deterioration occurring in patients with Alzheimer's disease is associated with progressive loss of cholinergic neurons and consequent decline in levels of acetylcholine (Ach) in brain[3].

Serotonin deficiency increases the risk for heart disease, dementia and Alzheimer's. Previous research findings using *Zingibe roffcinalis*, Brahm rasayana and *Hibiscus sabdariffa* have displayed a link between memory improving effect and cholinesterase activity. *Griffonia's* seeds contain 20% 5-HTP.

In the present study, the ethyl acetate,ethanol extract of*D. gangeticum* significantly decreased the AChE level and enhance the serotonin levels in the mice whole brain homogenate, indicting its potential in the attenuation of severity of Alzheimer's disease.

In the present study was showed that ethyl acetate and ethanol extracts of *D. gangeticum* extract exhibited,

1. Decreased acetylcholinesterase enzyme levels

2. Enhanced serotonin levels

3. Ultimately improved memory of mice in both exteroceptive and interoceptive behavioral models.

During the process of learning and memory formation the brain undergoes a physical and chemical change which is called as synaptic plasticity. It shows involvement of various signal transduction pathways, induction of gene expression which results in formation of new synapses between nerve cells. This process undergoes a continuous remodeling with time and new experiences. The free radical theory of aging is one of the most popular, single mechanistic theories of aging, which discloses increased generation of free radical as the major cause of cellular damage. Such free radical-mediated damages are prevalent during aging which leads to age associated diseases like Alzheimer's disease (AD) and Parkinson's disease. Impairment of memory is the initial and most significant symptom.

Alzheimer's diseaseis associated with a decline in cognitive abilities. The most common cause of dementia in the elderly is probably Alzheimer's disease. Despite the

severity and high prevalence of this disease, the allopathic system is yet to provide a satisfactory antidote.

In the present study, *D. gangeticum* ethyl acetate and ethanol extract (200mg/kg and 200mg/kg) administered orally improved learning and memory of mice assessed by the behavioral models like Y-Maze, Morris Water Maze and also interoceptive model like estimation of acetylcholinesterase and serotonin activity in brain tissue by spectroflurimetric method compared to the scopolamine induced mice. (Disease control)

Thus a combination of anticholinesterase, serotonergic agonist, neuroprotective effects exhibited by *D. gangeticum* may all be eventually responsible for memory improving effect was observed in the present study.

8. CONCLUSION

The central cholinergic system & serotonergic system plays an important role in learning and memory. In the present study, *Desmodium gangeticum* roots extracts (200mg/kg of ethyl acetate and 200mg/kg of ethanol) administered orally improved learning and memory of mice assessed by the behavioral models like Morris water maze, Y-maze. In Scopolamine induced amnesia there is loss of memory.

The *D.gangeticum* ethanol extract & *D. gangeticum* ethyl acetate extract contains majorly flavonoids, alkaloids, pterocarpenoids and antioxidant property which may responsible for the anti-amnesic effect.

- The neurotransmitter (acetylcholinesterase and serotonin) estimation of ethyl acetate and ethanol root extracts of *Desmodium gangeticum* by *ex vivo* method was evaluated.
- The memory activity of ethyl acetate and ethanol root extracts of *Desmodium gangeticum* by *in vivo* (Morris water maze and Y maze) method was evaluated

Further studies can be carried out in the future to elucidate the other neurotransmitter are to evaluate and then mechanism of action, clinical studies may for carried out to establish its efficacy in humans.

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