EVALUATION OF ANTIAMNESIC ACTIVITY OF AYURVEDIC FORMULATION SARASWATHA GRITA IN SCOPOLAMINE INDUCED RAT MODEL

A Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

HARITHA P. H.

261825803

Under the guidance of

DR. DITHU THEKKEKKARA M. Pharm. Ph.D.,

Department of Pharmacology

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD

COIMBATORE – 641048

APRIL 2020

DR. A. RAJASEKARAN, M. Pharm, Ph.D.,

PRINCIPAL,

KMCH COLLEGE OF PHARMACY,

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE - 641 048,

TAMILNADU

CERTIFICATE

This is to certify that the dissertation work entitled "**Evaluation of Antiamnesic Activity of Ayurvedic Formulation Saraswatha grita in Scopolamine Induced Rat Model**" is a bonafide research work carried out by the candidate **HARITHA P.H.** submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology**, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020.

Date: DR. A. RAJASEKARAN, M.Pharm, Ph.D.,

Place: Coimbatore   Principal

DECLARATION

I do hereby declare that the dissertation work entitled "**Evaluation of Antiamnesic Activity of Ayurvedic Formulation Saraswatha grita in Scopolamine Induced Rat Model**" submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmacology, was done by me under the guidance of **DR. Dithu Thekkekkara, M. Pharm, Ph.D.,** assistant professor Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020.

Date: **HARITHA P.H.**

Place: Coimbatore   **(Reg. No: 261825803)**

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled "**Evaluation of Antiamnesic Activity of Ayurvedic Formulation Saraswatha Grita in Scopolamine Induced Rat Model**" submitted by the candidate **Reg No:261825803** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology** is a bonafide work carried out by the candidate at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu and was evaluated by us during the academic year 2019-2020.

Examination Centre: KMCH College of Pharmacy, Coimbatore-48

 Date:

 Place: **Coimbatore**

Internal Examiner   **External Examiner**

                                Convener of Examination

Dedicated to Almighty,

My Beloved Parents

& Brother

ACKNOWLEDGEMENT

It was indeed awesome to share Master studies with wonderful people. I wish to thank my loving friends **Kanupriya J., Treasa Pearl, Cuckoo, Reshma, Keerthana, Athira, Nayanthara, Solly and Henna.**

I take this opportunity to extend my indebtedness, gratitude to all my juniors for their memorable company and involvement – especially to **Vishali, Kavitha, Pavithra, Jayanthi, Saranya, Nadhiya, Nivetha, Nishanth, Nijanthan, Rajesh, Raj** and so on…

I express my special thanks to **Mrs. Muneeshwari (Lab technician, Dept. of Pharmacology)**, **Mr. Tamilarasan (Lab technician, Dept. of Pharmacology), Mr. Saravanan (Lab technician, Dept. of Pharmacology), Mrs. Sudha (Dept. of Pharmacognosy), Ms. Jeeva (Dept. of Pharmaceutics)**, Librarian and other Lab technicians of KMCH College of Pharmacy for their valuable support and timely help during the course of entire work.

I have great gratitude to **Mrs. Dhanalakshmi** for helping in animal maintenance during the study.

I would like to express my whole hearted gratitude to my acha **Mr. Haridas P.S.** , my amma **Anitha Haridas** and my brother **Arundas P.H.** who remembered me in their prayers for the ultimate success. They gave me incessant moral support, encouragement and motivation in my academical pursuits to realise my dreams. My parents – my two life lines supported me financially and thus enabled me to concentrate fully on my studies without any worries.

Last but not least, I would like to thank each and everyone who was important to the successful realisation of this thesis.

ABBREVATIONS

ABBREVIATIONS	FULL FORM
%	Percentage
Ach	Acetylcholine
BBB	Blood Brain Barrier
CAT	Catalase
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals
DA	Dopamine
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
G	Gram
GABA	Gamma Amino Butyric Acid
5-HT	5- Hydroxytriptamine
IAEC	Institutional Animal Ethical Committee
i.p.	Intraperitonial
LPO	Lipid peroxidation
mg/kg	Milligram per kilogram
Nm	Nanometer
OECD	Organisation for Economic Corporation and Development
PBS	Phosphate buffer solution
p.o	Per oral
ROS	Reactive Oxygen Species
Rpm	Rotation per minute
SD	Standard deviation
Sec	Second
SEM	Standard Error Mean
SG	Saraswatha Ghrita
SOD	Super Oxide Dismutase
TBA	Thio barbituric acid
TCA	Trichloro Acetic acic
µg/mg	Microgram per milligram
w/v	Weight per volume

1. Introduction

Memory is extremely complex in terms of the kind of information that is represented in the brain, the processes associated with it, and its distribution across a variety of neural systems. Memory includes storing, retaining, and later recalling information. Memory consolidation and recall are dependent upon complex mnemonic mechanisms, which vary based on the type of memory representation [1]

1.1 Types of memory

There are mainly 2 types of memory

- Short term memory (STM) (Fig. no. 1)
- Long term memory (LTM) (Fig. no. 2)

Short-Term (working) memory

```
                    Elaborate rehearsal                    Maintenance rehearsal

   ┌──────────────┐  ┌──────────────┐          ┌──────────────┐  ┌──────────────┐
   │ Spatio-Visual│  │Spatio-Temporal│         │  Auditory    │  │  Articulate  │
   │  Rehearsal   │  │  rehearsal   │          │  rehearsal   │  │  Rehearsal   │
   └──────────────┘  └──────────────┘          └──────────────┘  └──────────────┘

                        ┌────────────────────────────┐
                        │  Short – Term episodic buffer │
                        └────────────────────────────┘
```
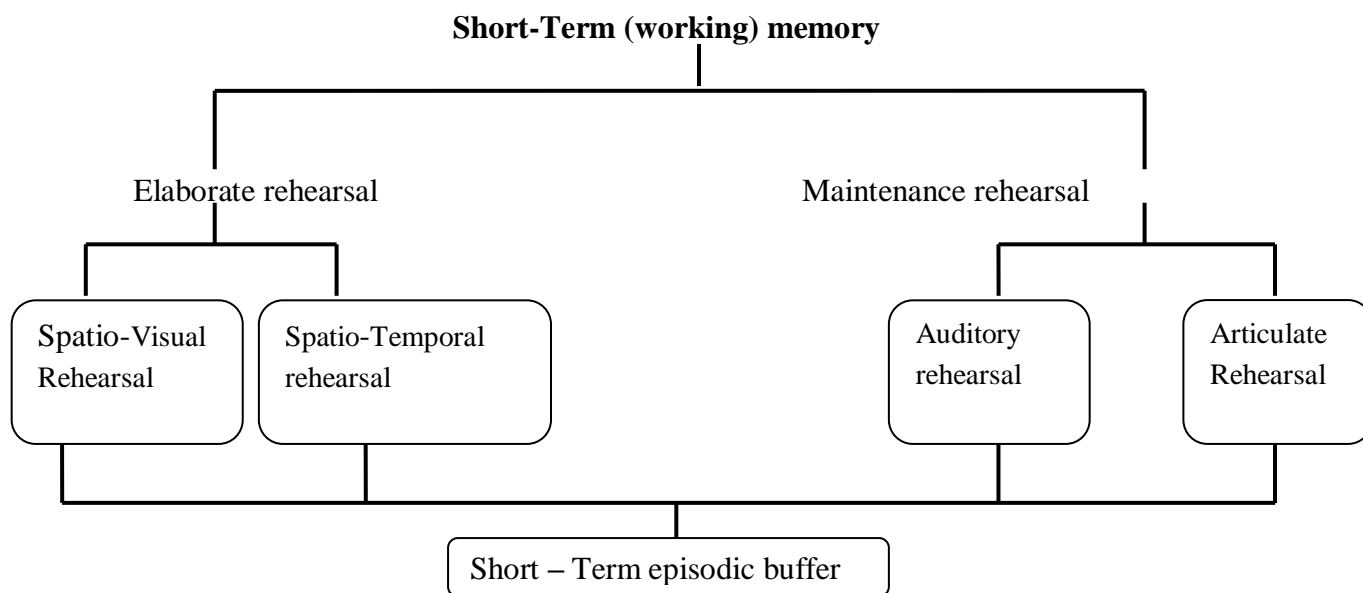
Fig. no. 1 : Various types of short term memory

STM is an essential component of cognition and is defined as the maintenance of information over a short period of time (seconds). STM can remain unimpaired in amnesic patients who show distinct LTM impairments. STM can be impaired while LTM functions remain intact. STM (primary memory) involves a conscious maintenance of sensory stimuli over a short period of time after which they are not present anymore. On the other hand,

LTM (secondary memory) involves the reactivation of past experiences that were not consciously available between the time of encoding and retrieval. STM engages repeated excitation of a cellular compound, LTM leads to structural changes on the synaptic level, which are preceded by consolidation processes that are thought to be highly dependent on hippocampal functions [2].

LTM refers to the mechanism by which acquired memories gain stability or are strengthened over time, and become resistant to interference. Successful long-term storage includes several steps starting with the encoding of information, followed by short-term storage and consolidation from STM to LTM, as well as repeated reconsolidation. Consolidation is thought to occur in a structured way allowing for prompt and precise retrieval. During consolidation, memories can undergo changes that can be quantitative (enhancement, strengthening) as well as qualitative in nature (e.g., awareness of underlying sequences) [3].
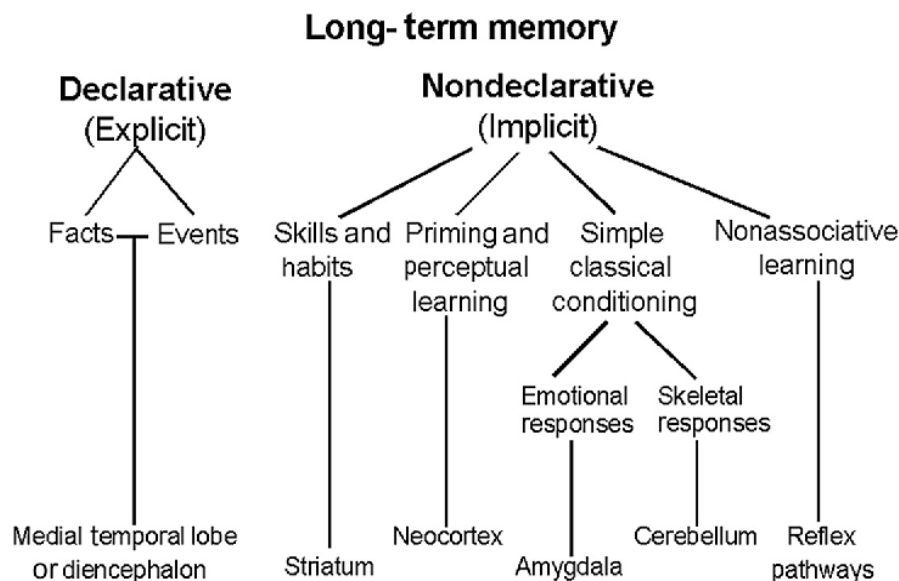

Fig. no. 2 : Various types of long term memory

The mnemonic processes underlying memory are multifaceted in that information is distributed across a variety of neural systems. On the other side, amnesia is characterized by the profound loss of memory in the presence of relatively preserved cognitive abilities [3].

Amnesic cases show impairments in mentally constructing scenes, navigating spatial layouts, and recalling and/or reconstructing previously experienced scenes [4].

1.2 Different Structures in the brain handle different kinds of memory

1.2. a. Hippocampus

The hippocampus and the adjacent entorhinal and perirhinal cortices are referred to as the hippocampal complex. The components of the hippocampal complex are highly interconnected by means of recurrent neuroanatomical circuits. Anatomically, the system is in a position to create integrated records of various aspects of memory experiences, including visual, auditory, and somatosensory information. The principal function of the hippocampal complex is the acquisition of new factual knowledge. With respect to the nature of the amnesia associated with hippocampal damage. Specifically, damage to the left hippocampal system produces an amnesic syndrome that affects verbal material (such as spoken words or written material) [5].
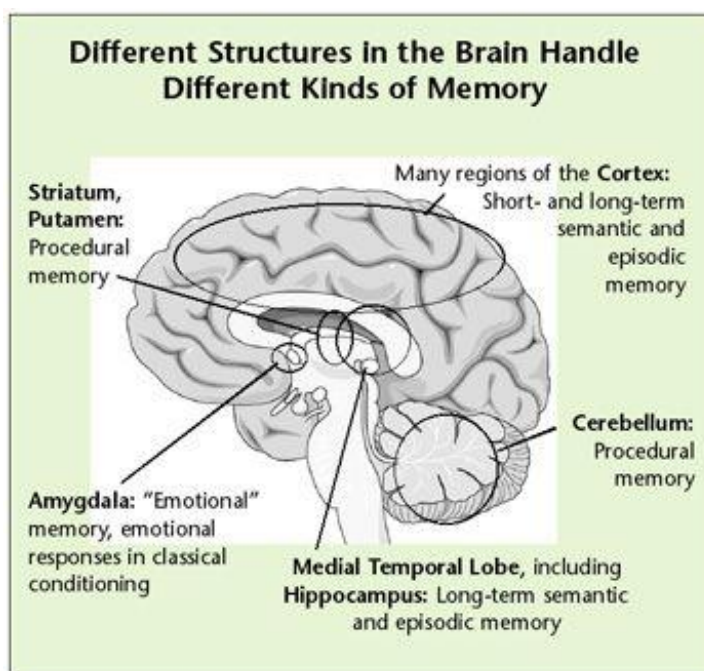
Fig. no. 3: Various parts brain handling different types of memory

1.**2**. **b.** <u>**Amygdala**</u>

The amygdala plays an intriguing role in memory. The amygdala is important for the acquisition and expression of emotional memory, but not for neutral memory. Specifically, the amygdala contributes critically to the potentiation of memory traces for emotional stimuli during their acquisition and consolidation into long-term declarative memory [6].

1.**2**. **c.** <u>**Frontal lobes**</u>

The frontal lobes make up nearly one-half of the entire cerebral mantle, and they represent the highest level of neural evolution. The cognitive operations mediated by the frontal lobes, such as foresight, complex decision-making, and social conduct, stand at the zenith of evolution of mental processes. An important function subserved by the dorsolateral prefrontal region is working memory. Working memory refers to a brief window of mental processing—on the order of a minute or two—during which a limited amount of information is held in a sort of mental scratchpad, and operations can be performed on it. Working memory is used to bridge temporal gaps, so that we can perform operations on material that is being held "in-mind" but is no longer existent in our perceptual space [7].

1.**2**. **d.** <u>**Subcortical structures**</u>

Many subcortical structures participate in various aspects of higher-order cognition and behavior. There are some subcortical structures, include the basal ganglia, thalamus, cerebellum, and brainstem nuclei.

The basal ganglia are a collection of gray matter nuclei deep in the brain, comprised mostly by the striatum, which includes the caudate nucleus and the putamen, and the pallidum, which includes the globus pallidus. The striatum and pallidum play basic roles in motor behavior, particularly in the automatic execution of highly learned motor patterns. In addition to their role in automatic motor behaviors, the striatum and pallidum also make important contributions to some aspects of higher-order cognition and behavior [5].

1.3 Amnesia

Amnesia is of two types,

- Anterograde
- Retrograde amnesia.

Anterograde amnesia (AA) refers to an impaired capacity for new learning. Retrograde amnesia (RA) refers to the loss of information that was acquired before the onset of amnesia. It has long been recognized that AA and RA tend to occur together in the same patients. In addition, the severity of AA is usually correlated with the severity of RA. Yet it is also true that RA can sometimes appear disproportionately severe in comparison to AA. Moreover, AA can sometimes occur in the absence of RA.

As the severity of AA increased, so did the severity of RA. Patients with damage to both the hippocampus and parahippocampal gyrus exhibited the most severe AA and the most severe RA. Patients with damage limited largely to the hippocampus exhibited less severe AA and less severe RA [6].

1.4 Reasons for memory loss/ amnesia

Selective damage to a number of brain regions has been associated with amnesia, including a circuit comprising the hippocampus, the diencephalon and the fibers connecting them [3].

Bilateral damage to the medial temporal lobe or diencephalic midline can cause memory impairment without affecting other cognitive functions. The brain lesions can produce highly specific cognitive deficits exquisitely related to the structures and connections that are damaged.

The medial temporal lobe, like other brain structures, is involved in processing and analyzing, but damage to this structure results in a memory deficit because the normal contribution of the structure is not essential until some time has passed after learning. The medial temporal lobe is involved in memory for a limited period of time after learning. As time passes, memory is gradually reorganized (or consolidated), and information storage in neocortex becomes independent of the medial temporal lobe system [7].

Hippocampus is implicated in spatial memory, some spatial abilities are preserved in hippocampal amnesia and ageing. Specifically, schematic spatial representations remain intact, but often lack coherent detail and/or are limited in their ability to support

performance in novel task situations. This suggests that extra hippocampal regions can support schematic spatial memory, but that the hippocampus plays a central role in forming detailed spatial representations that can be flexibly expressed or reconstructed under a variety of task conditions to support efficient performance [1].

Previous research like BBB Model predicts that the hippocampus is necessary for the construction of a spatially coherent mental image of any remembered scene, including shifts in viewpoint that enable one to mentally play through events [8], which indicates that the hippocampus receives inputs from all sensory modalities (e.g., sight, sound, taste, touch, etc.), suggesting that one possible function of the hippocampus is to use sensory markers to demarcate a spatial location, allowing the hippocampus to more efficiently represent spatial information.

Thus, one function of the hippocampus may be to encode and separate events in space resulting in spatial pattern separation. Spatial pattern separation would ensure that new highly processed sensory information is organized within the hippocampus, which in turn enhances the possibility of remembering and temporarily storing one place as separate from another place.

The separation of events may occur via pattern separation of spatial information, so that spatial locations are separated from each other and spatial interference is reduced. It should be noted that the idea that the hippocampus supports context as well as spatial arrangement of objects is consistent with the need to separate objects and/or other cues to achieve optimal spatial representations. The hippocampus utilizes spatial pattern separation to orthogonalize sensory input information and generate differential spatial (metric) representations.

Pattern association is the ability to form arbitrary associations between multiple items. It should be noted that it is also possible to consider the demonstration of arbitrary associations for multiple items as a form of cross-modal pattern separation based on multiple dimensions (e.g. objects and places) [1].

Ageing is also associated with deficits in spatial memory [4]. Pharmacological data demonstrate that the central muscarinic receptor antagonists impair performance on memory tasks and cause an amnesia-like syndrome in both rodents and primates [8]. The

characteristic feature of the human amnesic syndrome is a profound memory loss for events that occur subsequent to brain insult (ante- rograde amnesia, AA). Memory loss for pre-morbid events or retrograde amnesia (RA) is more variable, ranging from very little in some cases to considerable amounts that can extend back several decades. Investigations of memory loss in brain- damaged animals have confirmed that lesions to the hippocampal system or the dorso- medial thalamus produce severe AA, although the precise nature of the deficits may differ in the two cases [9].

1.5 Current treatment for amnesia

Any treatment or intervention designed to reverse amnesia,whetherchronic oracute, needsto also betested in control wild-type animals. This is crucial to account for general cognitive effects (e.g. arousal, attention, emotional response, etc.) that might affect behavioural performance independently of any improvement of memory engram function [10].

Treatment focuses on therapies and techniques that help improve quality of life which includes,

- vitamin B1 supplements, in case of a deficiency

- occupational therapy

- memory training

- technology assistance, such as reminder apps

There are currently no FDA-approved medications to treat amnesia.

1.6   Various *in vivo* models for amnesia

1.6 a. Amyloid Beta-Peptide-Induced Memory Impairment

The formation of amyloid-beta (Aβ) plaques are distinct characteristic features of AD and administration of Aβ peptide has been renowned for inducing memory loss [11]. The main form of Aβ consists of 40 amino-acid residues. These long forms of Aβ aggregate more efficiently as compared to the 40-amino-acid peptide. It is generally supposed that aggregated Aβ is accountable for progressive nature of disease, as the unregulated buildup

of aggregates is toxic to the brain [12]. Aβ-peptide-mediated inhibition of choline acetyl transferase (ChAT) to induce dysfunction of cholinergic neurons is one of the hallmark mechanisms of Aβ-peptide to induce AD [13]. Aβ augments hyperphosphorylation of tau and stimulates the development of neurofibrillary tangles in various cellular and animal models [14]. It has been established that continuous infusion or acute injection of Aβ into the brain leads to brain dysfunction followed by neurodegeneration and impairment of learning and memory very similar to that seen in AD [15].

1.6 b. Streptozotocin-Induced Memory Impairment

Streptozotocin (STZ), chemically is a glucosamine nitrosourea obtained from a soil microbe Streptomyces achromogenes [16]. ICV injection of STZ at a sub-diabetogenic dose (3 mg/kg), twice at an interval of 48 h in rodents elicits a progressive loss of memory seen very analogous to that of AD [17]. It also has the ability to generate neuronal damage by oxidative stress via the generation of ROS and reactive nitrogen species [18], increase in the levels of malondialdehyde, accumulation of Aβ in the brain, hyperphosphorylation of tau protein, as well as negative regulation of the genes linked to insulin signaling such as IGF-1 receptors [19].

1.6 c. Quinolinic Acid-Induced Memory Impairment

Quinolinic acid (QA), is a well known NMDA receptor agonist frequently used to induce HD in various experimental models [20]. QA administration produces many neurochemical and histopathological trends of HD neuropathology and also causes memory deficits [21]. Particularly, neurons within the hippocampus, neocortex and striatum are sensitive to QA but cerebellar, and spinal cord neurons are less sensitive. These variations in regional sensitivity most probably relate to variations in the configuration of NMDA receptors [22]. QA has been observed to augment the release of glutamate by neurons, slow down its uptake by astrocytes and restrain the activity of glutamine synthetase in astroglial cells leading to disproportionate glutamate concentrations and neurotoxicity [23]. It also has another mechanism of neurotoxicity like lipid peroxidation [24].

1.6 d. Colchicine-Induced Memory Impairment

Colchicine is a potent cytotoxic agent which causes depolymerization and inhibition of microtubules by irreversibly binding to itIt has been documented that central administration of colchicine produces memory deficits in rodents by cholinergic neurodegeneration and oxidative stress [25]. Colchicine inhibits axonal transport and augments neurofibrillary degeneration. Colchicine causes hippocampal lesions leading to learning and memory deficits, a decrease in ChAT, indicating that it could be used as an appropriate model for studying AD [26].Colchicine can provoke neurotoxicity and memory impairments by the destruction of cholinergic pathways, loss of cholinergic neurons, and reducing cholinergic turnover mainly in the hippocampus area of the brain [27].

1.6 e. Scopalmine induced amnesia model in rodent

There is evidence both from animal and human studies indicating that learning and memory can be modified by drugs which affect central cholinergic functions. For instance, scopolamine, a muscarinic receptor blocking drug, has been shown to impair memory, whereas muscarinic agonists, such as oxotremorine or arecoline, and acetylcholinesterase inhibitors, such as physostimnine, facilitate cognitive processes in animals and humans [28]. Administration of scopolamine produces deficits on tests of visual recognition memory, visuospatial praxis, verbal recall, visuospatial recall, psychomotor speed and visuoperceptual function [29]. Scopolamine induced memory impairment is one of the most widely used model because complex surgical procedures are not required. Amnesia can be induced in rats in the passive avoidance paradigm by administration of scopolamine, a central muscarinic receptor antagonist [30]. It has been accounted that scopolamine nonselectively blocks the binding sites of acetylcholine (ACh) muscarinic receptors in the cerebral cortex and consequences in disproportionate release of ACh which destroys the hippocampus nerves and causes impairment in learning and memory in a dose-dependent manner in mice [31]. Recently it is been found that there is a possible involvement of N-methyl-D-aspartate (NMDA) receptor mechanisms of dorsal hippocampus and/or septum in scopolamine-induced memory impairment [32].

So, amnesia is being treated with nootropic agents such as cholinesterase inhibitors to improve memory, mood and behavior, but the side effects associated with these agents have made their use limited. Ayurvedic medicine is based on the holistic view of treatment which promotes and supports equilibrium in different aspects of human life: the body, mind and soul. In Ayurveda medicinal plants and formulations are routinely used to slow down brain aging and enhance memory with less side effects. In this context, Saraswatham ghrutham, an Ayurvedic formulation which contains 7 major ingredients, *Terminalia chebula* , *Zingiber officinale*, *Acorus calamus*, *Piper longum, Piper nigram, Cyclea peltata and Moringa oleifera* which already reported for free radical scavenging activity, neuro protective and anti inflammatory activity is one of the promising formulation for amnesiac condition has been explored in the present study.

2. Review of Literature

Several investigations have also been carried out on Anti amnesic properties of plant-based extracts/phytochemicals. In what follows, the work carried out on Anti amnesic medications in Ayureveda and pharmacological activities of different ingredients of Saraswatha grita. The review is limited to 2018-1972.

Ayodele Jacob Akinyemi *et al.,* **(2018)** investigated the effect of essential oils from Nigeria ginger and turmeric rhizomes on some cytokines in cadmium (cd) induced neurotoxicity. The study revealed that essential oil from ginger and turmeric rhizomes exerts anti-infammatory efect by preventing alterations of some cytokines/inflammatory biomarker levels, inhibits the acetylcholinesterase (AChE) and adenosine deaminase (ADA) in Cd treated rats. They concluded that essential oil from *ginger and turmeric rhizomes* exerts anti-infammatory properties in Cd induced neurotoxicity [33].

Renente Rondina II *et al.,* **(2017)** studied the Evidence from amnesia and ageing inform the organization of space and time in hippocampal relational representations. They checked the oscillatory dynamics and suggested that relational information is organized hierarchically in memory, and temporal relations provide the foundation [2].

Yuh-Chiang Shen *et al.,* **(2017)** determined the neuroprotective effect and mechanisms of action underlying the *Terminalia chebula* extracts and ellagic acid by using beta-amyloid induced cell toxicity in an undifferentiated pheochromocytoma (PC12) cell line. They were evaluated the cell toxicity and changes in intracellular reactive oxygen species (ROS) and calcium level to evaluate the neuroprotective effects of *T.chebula*. They were concluded *T. chebula* extracts e.g., ellagic acid is crucial to verify the neuroprotective efficacy [34].

Hamid R. Sadeghnia *et al., (2017)* has studied protective effect of T. chebula alcoholic extract (TCAE) on oxidative PC12 and OLN-93 cells death induced by (Quinolinic acid) QA. The cells were pretreated with TCAE (6.25-50 μg/mL) for 2 h and then subjected to QA (8 mM) for 24 h and done the assay. Finally they found out that TCAE exhibits neuroprotection and oligoprotection potential by means of alleviating oxidative stress parameters [35].

Chatchada Sutalangka *et al.,(2017)* Determine the effect of the combined extract of Cyperus rotundus and Zingiber officinale (CP1) on the improvement of age-related dementia in rats with AF64A-induced memory deficits. Male Wistar rats weighing 180-200 g were orally given CP1

at doses of 100, 200 and 300 mg.kg for a period of 14 days after bilateral intracerebroventricular administration of AF64A. Spatial memory was assessed in all rats every 7 days throughout the 14 day-experimental period. At the end they concluded that CP1 is a potential novel food supplement for dementia [36].

Bing Wang *et al.,(2017)* studied the anti-inflammatory activity and chemical composition of dichloromethane extract from Piper nigrum and P. longum on permanent focal cerebral ischemia injury in rats. After subjecting the rats to permanent middle cerebral artery occlusion (pMCAO) for 6 h, at doses of 100 and 200 mg/kg, dichloromethane fraction from white pepper and long pepper, respectively, was intragastrically administered once a day for seven consecutive days. Cerebral cortical and hippocampal tissues were collected after seven days. Then they studied various neurochemicals and enzymes. And the results showed that dichloromethane fraction provides protection against cerebral ischemia [37].

Moses B. Ekong *et al.,(2017)* investigated the neuroprotective effects of Moringa oleifera (MO) leaf extract on aluminium-induced temporal cortical degeneration in rats. 24 male albino Wistar rats were grouped (n = 6) into control (1 ml/kg distilled water), l00 mg/kg aluminium chloride (AlCl3), 300 mg/kg MO, and 100 mg/kg AlCl3 and 300 mg/kg MO groups. Thebraintissueswerethenroutinelyprocessedfor some histological and immunnolabelling studies. Finally they cocluded that MO protects against Al-induced neurotoxicity of the temporal cortex of rats [38].

Hao-Long Liu *et al.,(2015)* developed effective and suitable UFLC–ESI-MS/MS method to simultaneously determine five characteristic constituents (piperine, piperlonguminine, Da,b-dihydropiperlonguminine, pellitorine and piperanine) of Piper longum L. The total alkaloids of P. longum L. was prepared. The alkaloid contents of Piper nigrum L. and P. longum L. were compared. The analysis was carried out in multiple reaction monitoring scan mode. And they cocluded that it's alkaloids have high neuroprotective activity [39].

Seyed Fazel Nabavi *et al.,(2015)* made a review article to provide a review of the available literature regarding the neuroprotective effects of luteolin and its molecular mechanisms of action. Luteolin is an important flavone, which is found in several plant products, including broccoli, pepper, thyme, and celery. Finally they presented convincing evidence for the potent antioxidant activity of luteolin in several in vitro and in vivo assay systems. Luteolin also suppresses inflammation in the brain tissues and regulates different cell signaling pathways [40].

Alyne Oliveira Correia et al., (2015) evaluated the neuroprotective activity of *Piper Nigram(pip)* in a model of PD. Male Wistar rats were grouped .All animals were subjected to behavioral studies, neurochemical, histological and immunohistochemical analyses. Based on the results they concluded *PIP* presented a neuroprotective action, probably a consequence of its antiinflammatory and antioxidant properties, making the drug a potential candidate for the treatment of neurodegenerative diseases as PD.

Sabrina Giacoppo *et al.,(2015)* made a review on the current knowledge about the neuroprotective effects of ITCs in counteracting oxidative stress as well as inflammatory and apoptotic mechanisms, using in vitro and in vivo models of acute and chronic neurodegenerative disease. Isothiocyanates (ITCs), derived from the hydrolysis of the corresponding glucosinolates (GLs), mainly found in Brassica vegetables (Brassicaceae) and, to a lesser extent, in Moringaceae plants, have demonstrated to exert neuroprotective properties. Therefore they cocluded in this review that ITCs, derived from the hydrolysis of corresponding GLs, could be promising compounds with neuroprotective effects in preventing and/or treating disorders related to nervous system at least in association with current conventional therapies [41].

Meena J *et al.,* (2015) assessed the *Cyclea peltata* which has medicinal plant in the folk medicine is used in this study to analyse its antioxidant potential. Swiss albino mice were subjected to toxicity analysis on treatment with methanolic extract of the plant after inducing them with DAL cells. The antioxidant levels on the liver and kidney tissues revealed lower ranges in control group when compared to the treated groups. Based on The results they concluded that methanolic extract showed a reversed value towards the normal.

Shaahin Harandi *et al.,* (2015) studied the Antiamnesic Effects of Walnuts Consumption on Scopolamine-Induced Memory Impairments in Rats. They mainly investigated the effects of walnuts consumption (2%, 6% and 9% walnut diets) on memory enhancement and acetylcholinesterase (AChE) activity of brain in scopolamine-induced amnesic rats. Finally they concluded that that walnuts may be useful against memory impairment and it may exert these anti-amnesic activities via inhibition of AChE activity in the brain [42].

Sivaraman Dhanasekaran *et al.,* (2015) carried out a study on In-vitro Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of Ipomoea aquatica Forsk: therapeutic lead for Alzheimer's disease. The main objective of the present study was to evaluate AChE inhibition and antioxidant activity of the plant Ipomoea aquatica Forsk.

Finally they concluded that all four extract has shown promising acetylcholinesterase inhibition activity in hydro alcoholic extract reveals the best inhibition potential [43]. **Mohammed Saleem Ali-Shtayeh *et al., (2014)* *carried out* In-vitro screening of acet ylcholinesterase inhibitory activity of extracts from Palestinian indigenous flora in relation to the treatment of Alzheimer's disease. They partly aimed at investigating in vitro possible AChEIs in herbal medicines traditionally used in Palestine to treat cognitive disorders, and to point out the role of these plants as potential sources for development of newly potent and safe natural therapeutic agents of AD. Finally they have shown to be simple, accurate, sensitive, spectrophotometric and colorimetric, and superior to Ellman's, and therefore can be used efficiently for qualitative and quantitative studies of AChEI activities of extracts [44].

Atul Puri *et al .,* **(2014)** carried out a study on Scopolamine induced behavioral and biochemical modifications and protective effect of Celastrus *paniculatous* and *Angelica glauca* in rats. They treated the rats with various doses of drugs and assessed the changes in behavioral and biochemical parameters. Finally they concluded that combined treatment of C. paniculatous and A. glauca exhibit protective efficacy in scopolamine induced dementia and promising as a memory enhancing agents that is associated with its strong antioxidant potential [45].

Md. Abdul Hannan *et al.,(2014)* conducted a study to evaluate the effect of Moringa oleifera leaf in the primary hippocampal neurons regarding its neurotrophic and neuroprotective properties. At the end they concluded that MOE promotes the development of primary hippocampal neurons by accelerating the rate of neuronal differentiation, increasing both axonal and dendritic length and branching, and modulating synaptic connectivity, and also provides neuronal protection. [46].

Lucian Hritcu *et al.,(2014)* analyzed the possible memory-enhancing and antioxidant proprieties of the methanolic extract of Piper nigrum L. fruits (50 and 100 mg/kg, orally, for 21 days) in amyloid beta(1–42) rat model of Alzheimer's disease. The memory-enhancing effects of the plant extract were studied by means of in vivo (Y-maze and radial arm-maze tasks) approaches. Finally they suggested that piperine, the main alkaloid of black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit-like condition of AD and also could serve as the potential functional food to improve brain function [47].

T. I. Rasyidah *et al.***, (2014)** investigated the possible antioxidant activity of *Zingiber officinale (ginger)* ethanolic extract on formalin-induced testicular toxicity in rats. *in vivo* antioxidant studies were assessed upon harvested testicles. They measured MD, SOD, CAT levels as compared to control. Based on the results they concluded the *ginger* exhibit antioxidant properties which proven by the increase of SOD and CAT activities [48]

Hee Ju Kim *et al.,* **(2014)** performed this study to investigate the autophagy enhancing effect of chebulagic acid on human neuroblastoma SH-SY5Y cell lines. They determined the effect of chebulagic acid on expression levels of autophagosome marker proteins such as, DOR/TP53INP2, Golgi-associated ATPase Enhancer of 16 kDa (GATE 16) and Light chain 3 II (LC3 II), as well as those of its upstream pathway proteins, AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR) and Beclin-1. All of those proteins were modulated by chebulagic acid treatment in a way of enhancing the autophagy. And they concluded that chebulagic acid is an attractive candidate as an autophagy-enhancing agent and therefore, it may provide a promising strategy to prevent or cure the diseases caused by accumulation of abnormal proteins [49].

ChatchadaSutalangka *et al.,(2013)* determined the antioxidant and nootropic activities of Moringa oleifera, the enhancement of spatial memory and neuroprotection of M. oleifera leaves extract in animal model of agerelateddementia Based on the crucial role of oxidative stress in age-related dementia. MaleWistarrats,weighing180–220g, were orally given M. oleifera leaves extract at doses of 100, 200, and 400mg/kg at a period of 7 days before and 7 days after the intracerebroventricular administration of AF64A bilaterally. Then, they were assessed memory, neuron density, MDA level, and the activities of SOD, CAT, GSH-Px, and AChE in hippocampus. Finally they suggested that M.oleifera leaves extract is the potential cognitive enhancer and neuroprotectant [50].

ChiaLinChang *et al.***, (2012)** carried out a study to determine phytochemical compositions, chemiluminescence antioxidant activities, and neuroprotective effects on PC12 cells for water, methanol, and 95% ethanol extracts of the air-dried fruit of Terminalia chebula Retzius. And they concluded that, the three extracts are new potential sources of natural antioxidants for food and nutraceutical products. The methanol and water extracts exhibit neuroprotective activities against H_2O_2-induced toxicity toward PC12 cells and are potential candidates for the treatment of H2O2induced neurodegenerative disease [51].

Anu Elizabeth Joy *et al.,* **(2012)** assessed anti catalytic activity of MO of Neuroleptic induced catalepsy is an effective animal model for screening the antiparkinsonian activity of a chemical. The results indicate that administration of haloperidol (1mg/kg, i.p) significantly induced catalepsy in Swiss albino mice, which was significantly reversed by the ethanolic extract of Moringa oleifera(200mg/kg i.p). They finally confirmed that anticataleptic activity of Moringa oleifera can be due to its antioxidant potential or due to its effect on brain monoamines [52].

Ghulam Moinuddin *et al.,* **(2011)** carried out a study on Modulation of Hemodynamics, Endogenous Antioxidant Enzymes, and Pathophysiological Changes by Angiotensin-Converting Enzyme Inhibitors in Pressure-Overload Rats. And finally they concluded that ACE-inhibition causes an improvement in myocardial antioxidant reserve, reduces oxidative stress, and prevents pathophysiological alterations, while showing a trend for potential target organ protection in hypertensive rats [53].

David Raj Chellappan *et al.,* **(2011)** conducted Acute oral toxicity study as per OECD-423 guidelines and the extract was found to be devoid of any conspicuous acute toxicity in extract treated animals and no mortality upto 2 g/kg by oral route. Hydroalcoholic extract of *C. peltata* in a dose dependant manner (125 and 250 mg/kg. *p.o.*) showed significant gastric protection against the ethanol-induced gastric ulcer model in rats [54].

Arunachalam Muthuraman *et al.,***(2011)** investigated the attenuating role of Acorus calamus plant extract in chronic constriction injury (CCI) of sciatic nerve induced peripheral neuropathy in rats. Hot plate, plantar, Randall Selitto, Von Frey Hair, pin prick, acetone drop, photoactometer and rota-rod tests were performed to assess degree of thermal, radiant, mechanical, chemical sensation, spontaneous motor activity and motor co-ordination changes respectively. Finally they cocluded that Acorus calamus prevented CCI induced neuropathy which may be attributed to its multiple actions including anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory actions [55].

Jintanaporn Wattanathorn *et al.,***(2011)** conducted a study on the elucidation of possible protective effect of Zingiber officinale, a medicinal plant reputed for neuroprotective effect against oxidative stress-related brain damage, on brain damage and memory deficit induced by focal cerebral ischemia. Wistar rats were administrated an alcoholic extract of ginger rhizome orally 14 days before and 21 days after the permanent occlusion of right middle cerebral artery (MCAO) and underwent the various studies. Finally they suggested that Z. officinale possessed

the protective effectagainstfocalcerebralischemia induced by the occlusion of right middle cerebral artery. It could attenuate the memory impairment, neurodegeneration, and brain infarct volume in this condition [56].

Kondeti Ramudu Shanmugam *et al.,(2010)* investigated the effect of ginger on oxidative stress markers in the mitochondrial fractions of cerebral cortex (CC), cerebellum (CB), hippocampus (HC) and hypothalamus (HT) of diabetic rats. A marked decrease in anti-oxidant marker enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH) and increase in malondialdehyde (MDA) was observed in the diabetic rats. At last they suggested that ginger exhibit a neuroprotective effect by accelerating brain anti-oxidant defense mechanisms and down regulating the MDA levels to the normal levels in the diabetic rats [57].

Raymond P. Kesnera *et al.,* **(2009)** carried out a study on Developing an animal model of human amnesia: The role of the hippocampus. And they found that notwithstanding, rats and humans with hippocampal damage are similarly impaired on analogous tasks that assess spatial memory, spatial pattern separation, spatial configural and arbitrary associations, temporal order memory, temporal pattern separation, sequential learning, and temporal associations. On the bisis this information they concluded that the rat can serve as a model of memory dysfunction (amnesia) as it relates to spatial and temporal processing of new information [1].

Fijesh P. Vijayan *et al.,* **(2007)** has evaluated the protective effect of a 70% methanolic leaf extract of *Cyclea peltata Lam* on cisplatin-induced renal toxicity. They measured the biochemical parameters in kidney tissues. The marked cisplatin-induced renal damage, characterized by a significant increased in biochemical enzymes level by the extract. They observed methanolic leaf extract could be used as a natural antioxidant against cisplatin-induced oxidative stress [58].

Pradeep K. Shukla *et al.,(*2006**)** investigated the neuroprotective potential of ethanol:water (1:1) extract of rhizomes of Acorus calamus in middle cerebral artery occlusion (MCAO)– induced ischaemia in rats. A significant behavioural impairment in Rota–Rod performance and grid walking was observed in rats, 72 hours after MCAO as compared to sham–operated animals. And the result of this study exhibited neuroprotective efficacy of A. calamus by modulating anti oxidant capacity in rats with MCAO [59].

Jungsook Cho *et al.,(2002)* evaluated neuroprotective actions and action mechanisms of the isolated asarone as well as the a- and the h-asarone obtained commercially. The isolated asarone inhibited the excitotoxicity induced by the exposure of cortical cultures for 15 min to 300 AM NMDA in a concentration-dependent manner and the commercially obtained a- and h-asarone exhibited more potent inhibitions of the NMDA-induced excitotoxicity than the isolated asarone. that asarone, the major essential oil component in AGR, exhibits neuroprotective action against the NMDA- or Glu-induced excitotoxicity through the blockade of NMDA receptor function [60].

Pradeep K. Shukla *et al.,(2002)* studied the Protective Effect of Acorus calamus Against Acrylamide Induced Neurotoxicity. Rats were exposed to acrylamide (ACR) caused hind limb paralysis in 58% of the animals on day 10 and decreased behavioural parameters, namely distance travelled, ambulatory time, stereotypic time and basal stereotypic movements compared with the control group. At the end they concluded that the neurobehavioural changes produced by ACR may be prevented following treatment with Acorus calamus rhizomes [61].

B. Topic *et al.,(2002)* assessed the effects of i.g. administration of Zingicomb (ZC), a mixture of zingiber officinale and ginkgo biloba extracts, on learning and memory, and on indicators of oxidative stress in aged rats. Effects of ZC (1 and 10 mg/kg) were investigated in 22–24 months old Wistar rats using the Morris water maze. Treatment was administered on days 3 and 4 of training, then over 7 days with training discontinued, and again on days 5 and 6 when training was resumed. Finally they found out that ZC seems to alleviate age-related spatial navigation deficits in old Wistar rats and secondly, chronic application of ZC for 5 months reduces the amount of markers of oxidative stress [62].

William S. Stone *et al.,* (1998) studied Attenuation of scopolamine-induced amnesia in mice. They examined whether amnesia induced by scopolamine could be counteracted in mice by arecoline, a cholinergic agonist, or by other drugs, epinephrine or glucose, which have been found to enhance memory in aged rodents and humans. Finally their results support the value of scopolamine as a model of age-related memory impairments, but suggest further that these memory deficits may be particularly susceptible to attenuation with non-cholinergic treatments [3].

Rakesh Kakkar *et al.,* (1995) studied the Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. They hypothesized that oxygen free radicals (OFRs) may be involved

in pathogenesis of diabetic complications. At the end they suggest that oxidative stress occurs in diabetic state and that oxidative damage to tissues may be a contributory factor in complications associated with diabetes [63].

Philippe Chopin *et al.,* **(1992)** studied the Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. And they concluded that amnesia can be induced in rats in the passive avoidance paradigm by administration of scopolamine, a central muscarinic receptor antagonist [4].

Margret Schlumpf *et al.,***(1974)** carried out A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Finally theyconcluded that 5-HT, NA and DA can be determined in two pieces (1.55 mg) of small corresponding areas from a single mouse or rat brain by fluorometry when the sensitivity of the method is increased by volume reduction throughout the entire assay procedure, in combination with some additional adjustments of reagent concentrations [64].

Hara P. Misra *et al.,* **(1972)** studied the The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. And they concluded that the ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme [65].

3. Aim and Objectives

3.1 Aim

The aim of the present study is to evaluate the anti amnesia activity of Ayurvedic formulation Saraswatha ghrita in scopolamine induced rat model.

3.2 Objectives

- To evaluate the anti-amnesic activity of ayurvedic formulation saraswatha ghrutha in scopolamine induced rats.

- To evaluate the endogenous anti oxidant enzyme level.

- To estimate neuro chemical parameters.

3.3 Table No: 1 Contents of Ayurvedic Formulation Saraswatha Ghrita

INGREDIENTS	QUANTITY IN EACH 10 G	REPORTED ACTIVITY
Terminalia chibula(P.)	0.313 g	Neuroprotective effect anti-inflammatory, antioxidant, anti-lipid peroxidative and membranestabilizing effects [66,67]
Zingiber officinale(Rz.)	0.313 g	Anti oxidant and neuroprotective [68,69,70]
Piper nigram(Fr.)	0.313 g	Anti inflammatory andantioxidant [71,72]
Cyclea peltata(Rt.)	0.313 g	anti-oxidative, anti inflammatory and

		neuroprotective [73,74,70]
Acorus calamus (Rz.)	0.313 g	anti-oxidative, antiinflammatory, and calcium inhibitory actions and neuroprotective effects [75,69]
Moringa oleifera(Rt.Bk)	0.313 g	Antioxidant anti catalytic and neuroprotectve [67]
Rock salt	0.310g	Anti oxidant and anti inflammation
Goat's milk	10.9 ml	Rejuvenator and probiotics
Ghee	10 g	Improve cognition

4. **Plan of Work**

- ➢ Induction of dementia by scopolamine
- ➢ Screening of behavioral studies
  - Morris water maze test
  - Open field test
  - Passive avoidance paradigm
- ➢ Endogenous antioxidant enzyme studies
  - Estimation of acetyl cholinesterase
  - Estimation of lipid peroxidation
  - Estimation of superoxide dismutase activity
  - Estimation of catalase activity
- ➢ Estimation of neuro transmitter levels
  - Estimation of dopamine (DA)
  - Estimation of serotonin (5-HT)
- ➢ Histopathological evaluation
- ➢ Data collection
- ➢ Statistical Analysis
- ➢ Interpretation and Discussion of Results

Animals were divided into six groups of six rats in each group.

- ➢ Group I   :   Vehicle control (0.5 % w/v Na CMC)
- ➢ Group II   :   Scopolamine only (2 mg/kg i.p)
- ➢ Group III   :   Scopolamine+ donepezil (2.5 mg/kg, p.o)
- ➢ Group IV   :   Scopolamine + ayurvedic formulation (200 mg /kg)
- ➢ Group V   :   Scopolamine + ayurvedic formulation (100 mg /kg)
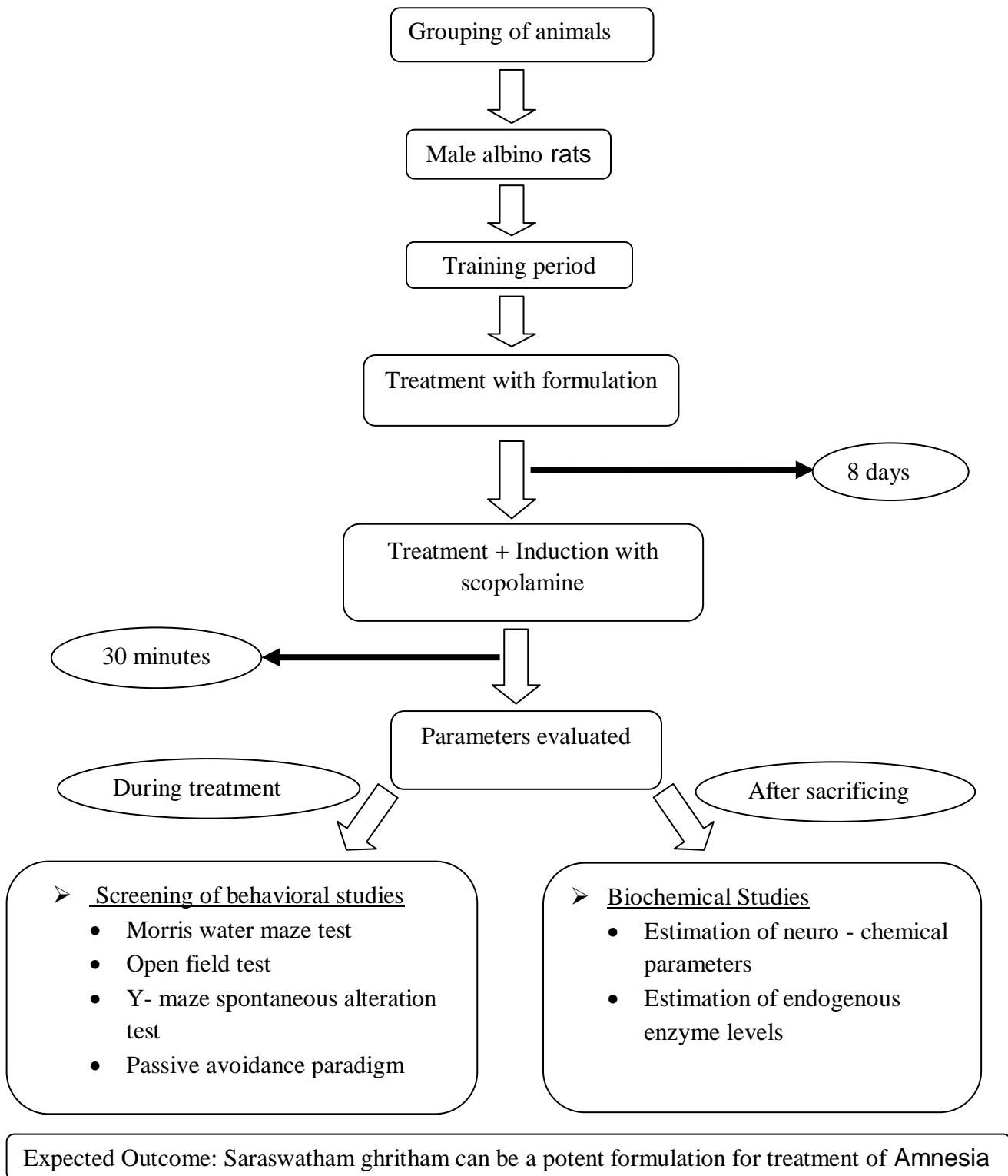
4.1 Schematic Representation

```
                    ┌─────────────────────┐
                    │  Grouping of animals │
                    └─────────────────────┘
                              ↓
                    ┌─────────────────────┐
                    │   Male albino rats   │
                    └─────────────────────┘
                              ↓
                    ┌─────────────────────┐
                    │   Training period    │
                    └─────────────────────┘
                              ↓
                ┌───────────────────────────┐
                │ Treatment with formulation │
                └───────────────────────────┘
                              ↓ ──────────────────→ ( 8 days )
                ┌───────────────────────────┐
                │   Treatment + Induction    │
                │     with scopolamine       │
                └───────────────────────────┘
         ( 30 minutes ) ←──────────────────  ↓
                    ┌─────────────────────┐
                    │  Parameters evaluated │
                    └─────────────────────┘
       ( During treatment )  ↙          ↘  ( After sacrificing )
```

- ➤ Screening of behavioral studies
  - Morris water maze test
  - Open field test
  - Y- maze spontaneous alteration test
  - Passive avoidance paradigm

- ➤ Biochemical Studies
  - Estimation of neuro - chemical parameters
  - Estimation of endogenous enzyme levels

Expected Outcome: Saraswatham ghritham can be a potent formulation for treatment of Amnesia

Fig. No: 4 schematic diagram of plan of work

5. Materials and Methods

5.1 Procurement of Formulation

Ayurvedic formulation saraswatham ghritam (SG) was procured from Vaidyaratnam P.S. varier's ARYA VAIDYA SALA, kottakkal, kerala, india in month of October-2019.

5.2 Standardization Of Ayurvedic Formulation

Physico- chemical studies like refractive index, specific gravity, acid value, saponification value, iodine value, determination of unsaponifiable matter, peroxide, viscosity, rancidity test and HPTLC were carried out as per the WHO guidelines, Ayurvedic Pharmacopoeia and Indian Pharmacopoeia [73,74,75] .

5.3 Methodology:

The studies were done at KMCH College of Pharmacy, Coimbatore, tamilnadu,India as per standard procedure.

5.3. a. Refractive Index:

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundry line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.33217 at 28˚C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3320, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

5.3. b. Specific Gravity:

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

5.3. c. Acid Value:

Weighed 2-10 g of ghritha in a conical flask. Added 50 ml of acid free alcoholether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

5.3. d. Saponification Value:

Weighed 2 g of the Amritaprasha ghritha into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

5.3. e. Iodine Value:

The sample was accurately weighed in a dry iodine flask. Dissolved with 10 ml of CCl4, 20 ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17 ℃ for 30 min. 15 ml of potassium iodide and 100 ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

5.3. f. Determination of Unsaponifiable Matter:

Weighed 5 g of the Amritaprasha ghritha into the flask. Added 50 ml alcoholic KOH into the sample. Boiled gently but steadly under reflux condenser for one hour. The condensor was

washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50 ml of water was added to the separating funnel followed by an addition of 50 ml petroleum ether. The stopper was inserted and shaken vigorously for 1 min and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50 ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25 ml of aqueous alcohol and shaked vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25 ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85 °C for about 1 h to remove the last traces of ether. A few ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desicator to remove last traces of moisture and then weighed.

5.3. g. Peroxide Value:

5 g of the Amritaprasha ghrita was weighed accurately into a conical flask, added 30 ml of mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5 ml of potassium iodide, allowed it to stand for 1 minute, add 30 ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5 ml of starch indicator continued the titration until blue color disappears.

$$\text{Peroxide value} = 10(a-b) / W$$

Where W= weight in g of the substance

5.3. h. Viscosity:

The given sample was filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary was vertical and the specified temperature is attained by the test liquid. The liquid

was sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured.

Viscosity is measured using the formula:

$$\eta_1 = \frac{\rho_1 t_1 \; X \; \eta_2}{\rho_2 t_2}$$

η_1 – Viscosity of sample

η_2 - Viscosity of water

t_1 and t_2 - time taken for the sample and water to pass the meniscus

ρ_1 and ρ_2 – Density of sample and water

X= Specific gravity of sample x 0.9961/specific gravity of water

η = Xx Time for samplex1.004/specific gravity of water x70sec

5.3. **i**. **Rancidity Test:**

1 ml of melted fat was mixed with 1ml of conc. HCl and 1 ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

5.3. **j**. **Sample Preparation for HPTLC:**

Sample obtained in the procedure for the determination of unsaponifiable matter was dissolved in 10 ml of chloroform this was followed for all the sample of Amritaprasha ghritha, and chloroform soluble portion was used for HPTLC. HPTLC: 4, 8 and 12 µl of the above sample of Amritaprasha ghrita was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in toluene - ethyl acetate (9:1) and the developed plates were visualized under short UV, long UV, and after derivatisation in vanillin-sulphuric acid spray reagent it was visualized under white light and

scanned under UV 254 nm, 366 nm and 620 nm. Rf , colour of the spots and densitometric scan were recorded.

5.4 Induction of Amnesia by Scopolamine

Saraswatha ghrita is given for total duration of 10 days while scopolamine was administered 30 min prior to behavioral paradigm in all the representative groups. Behavioral studies were carried out within the 24 h of last dose of treatment. For neurochemical assay animals were sacrificed by using ketamine and the brains are removed and wash with ice-cold isotonic saline, dissected into brain regions and used for the study.

5.5 Behavioral studies

5.5 a. Morris Water Maze Test

The test was carried out in a black circular pool (160 cm in diameter and 80 cm in height) filled with water to a depth of 40 cm (21±2 °C). The pool was divided into four equal quadrants. A platform (10 cm in diameter) was submerged 1.5 cm below the surface of the water in the center of one of the quadrants. The experiment was performed in a dimly lit room with some visual cues around the maze. Performance of each animal was monitored by a video tracing system. Twenty four hours prior to the start of the test, rats were habituated to the pool by allowing them to swim for 60 seconds in the absence of the platform. In this protocol, each rat accomplished three block sessions with interblock intervals of 30 min. Each block per sec, consisted of four successive trials with 60 seconds duration. On each trial, rats were randomly released into the water from one of the four quadrants facing the wall of the maze.

During acquisition, the location of the platform remained constant and rats were allowed to swim to the hidden platform. When animal found the platform, it was permitted to remain there for 30 seconds and then returned to its cage for a 30 second inter-trial interval. If the rat failed to find the platform within 60 seconds, it was guided toward the platform. The time spent and distance moved from the starting point to the platform were collected and analyzed later. Two hours later, spatial memory was examined with a single probe trial. During this trial, the platform was removed and the rats were allowed to swim for a 60 second period. The time spent and distance

moved in the quadrant where the platform was previously located were analyzed as a measure of spatial memory retention.

Following the probe trial, a visible platform test was performed for each rat to assess whether any motivational factors interfered with the rats' ability to escape to a visible platform. The platform was raised two cm above the water surface and became visible. The animal's ability to escape to the visible platform was evaluated by a blind observerd [76].

The water maze consist of a circular tank with 100 cm of diameter and a wall 20 cm above the water level

⇩

Training take place on 3 consecutive days, with the rats recieving four consecutive trials per day with an inter-trial interval of 6-10 minutes

⇩

Each trial was started from one of four assigned polar position with different sequence each day

⇩

The latency to find the platform is measured as the of placement of the rat in the water to time it find the platform

⇩

On day 4 a probe test was performed the platform was removed and the time spend in the target quaderant was measured .

⇩

Increases the latency to find the platform in the training period

⇩

The rat which solve the escape immediately are considered to have retain memory

Fig. No: 5 Flow chart of Morris water maze test

5.5 b. Open Field Apparatus

The open field test (OFT) is a common measure of exploratory behavior both qualitatively and quantitatively. Animals were removed from the home cage and placed directly into one corner of the open field (120cm×120cm). The floor was divided into a grid of 8×8 squares. Movement of the animal in the arena during the 10-minute testing session was recorded. After 10 minutes, the animal was removed and returned to the home cage, and the walls and floor surfaces were thoroughly cleaned with 5% ethanol between the tests to prevent olfactory cues from affecting the behavior of subsequently tested rats. Exploration was defined as the time spent in the inner 6×6 squares, whereas overall activity was defined as the number of squares crossed during the testing session. Although other parameter like distance in outer area grooming, latency stretch attend posture, latency of leave center area etc are measured [42].

Drugs were injected intra peritoneally 10-40mts before test.

Observed for 15 mints in a square field arena equipped with 2 rows of 8 photo cells, sensitive to light

Measurements were made in the dark in a ventilated, sound attenuating box

Number of rearing and crossings are measured
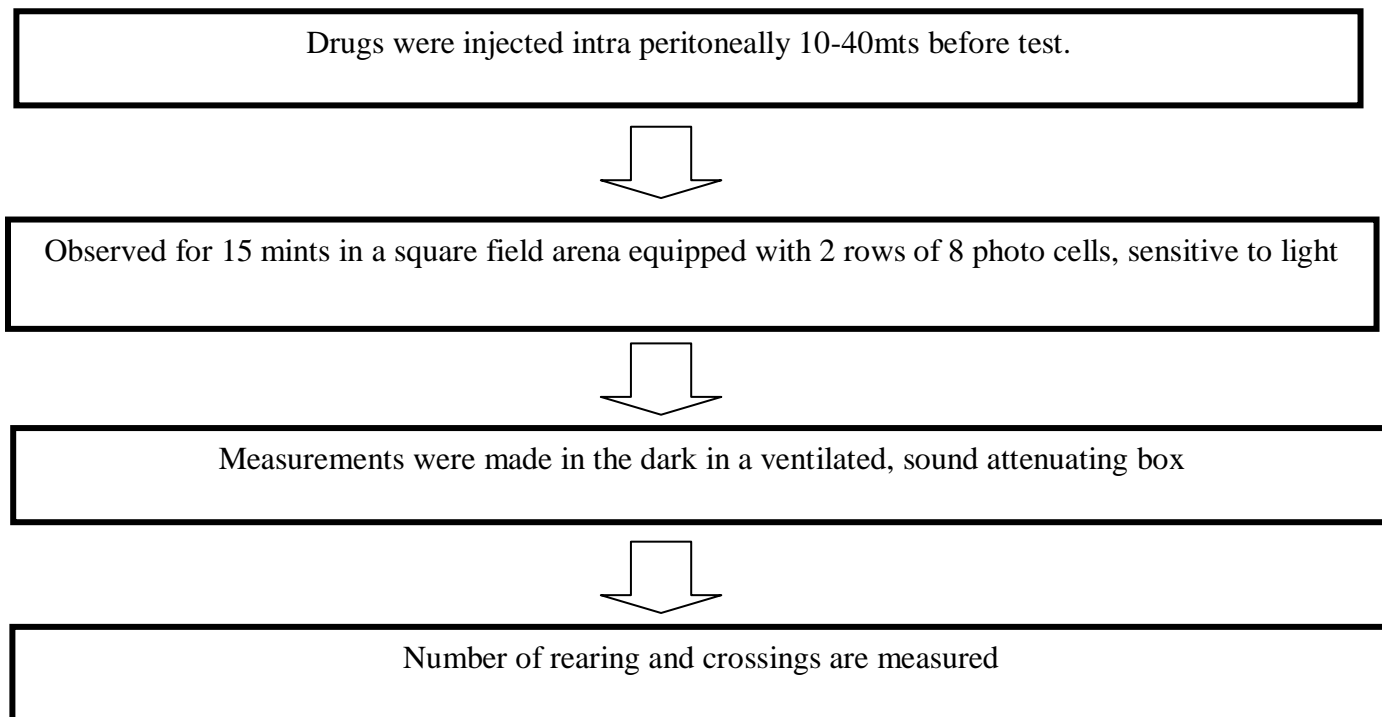
Fig. No: 6 Flow chart of open field test

5.5 c. Passive avoidance paradigm

This test is used to assess short-term or long-term memory on small laboratory animals. In this test, subjects learn to avoid an environment in which an aversive stimulus (such as a foot-shock) was previously delivered [45].
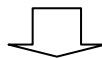
The rats were subjected to the passive avoidance test by placing in a light compartment of shuttle box.
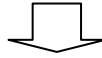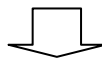
The light compartment was isolated from the dark compartment by a guillotine door.
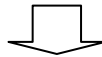
After an acclimatization period of 30 s, the guillotine door was opened and closed after entry of the rat into the dark compartment.

The subject received a low-intensity foot shock (0.5 mA; 10 s) in the dark compartment.

The transfer of the animal from one compartment to another was recorded as transfer latency time (TLT) in seconds.
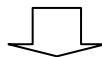
The duration of a trial was 270 s.

The first trial was for acquisition and retention was tested in a second trial given 24 h after the first trial. The shock was not delivered in the retention trials to avoid reacquisition.
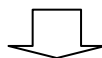
The criterion for learning was taken as an increase in the TLT on retention trials when compared with acquisition trial.

Fig. No: **7 Flow chart of Passive avoidance paradigm**

5.6 Histological Assessment

After behavioural and biochemical studies, the brains of different groups were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and post fixed in the same fixative overnight at 48°C. The brains were embedded in paraffin and stained with Hematoxylin-Eosin. The hippocampus lesions were assessed microscopically at 40 magnifications [77].

5.6 a. Fixation

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,

b) Prevents autolysis,

c) Preserves the structure of the tissue, and

d) Prevents shrinkage

Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining: Deparaffin the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

5.7 Acetyl cholinesterase Inhibition Assay

Principle

AChE activity was measured by using spectrophotometer based on Ellman's method with some modifications. The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2- nitrobenzoate which can be detected at 412 nm [43].

.**Procedure**

- 3 mL of reaction mixture, 2.6 mL of phosphate buffer, pH 7.2, 100 μL of the test samples or donepezil (1.25-40 μg/mL), 100 μL of 75 mM ATCI (dissolved in buffer), and 100 μL of 10 mM 5,5'-DTNB.

- The reaction was then initiated by the addition of ATCI. The concentration of DMSO in final reaction mixture was < 1%.

- The hydrolysis of ATCI was monitored by the formation of yellow 2-nitro-5-sulfidobenzene-carboxylate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine

- Measured at a wavelength of 412 nm using Spectrophotometer [43,44].

5.8 Estimation of Brain Neurotransmitter

Preparation of tissue extracts

Reagents

- HCl – Butanol solution: (0.85 ml of 37% hydrochloric acid in one-litre n-butanol)

- Heptane

- 0.1 M HCl: (0.85 ml conc. HCl up to 100 ml H2O)

. **Procedure**

At the end of experiment, rats were sacrificed and the whole brain was dissected out. 0.25 g of tissue was weighed and was homogenized in 5 mL HCl–butanol with motor driven Teflon coated homogenizer for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 mL) was removed and added to centrifuge tube containing heptane (2.5 mL) and 0.1 M HCl (0.31 mL). 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 was then taken either for 5-HT or NA and DA assay.

5.8 a Estimation of dopamine

Reagents

- 0.4 M HCl: 0.34 ml conc. HCl up to 10 mL H2O
- Sodium acetate buffer (pH 6.9): 0.72 mL of 1 M acetic acid (6 μL of glacial acetic acid up to 1000 μL with distilled water) + 6.84 mL of 0.3 M sodium acetate (0.408 g of sodium acetate in 10 mL distilled water) and volume were made up to 25 mL with distilled water. pH was adjusted with sodium hydroxide solution.
- 5 M sodium hydroxide: 5 g of NaOH pellets dissolved in distilled water and volume was made up to 25 mL with distilled water.
- M Iodine solution (in Ethanol): 1 g of potassium iodide + 0.65 g of iodine dissolved in ethanol and volume was made up to 25 mL.
- Sodium thiosulphate solution: 0.625 g Na2SO3 in 2.5 mL H2O + 22.5 mL 5 M NaOH
- 10 M Acetic acid: 14.25 mL of glacial acetic acid dissolved in distilled water and made up to 25 mL.

Procedure

To 1 mL of aqueous phase, 0.25 mL 0.4 M HCl and 0.5 mL of Sodium acetate buffer (pH 6.9) were added followed by 0.5 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by the addition of 0.5 mL Na2SO3 solution. 0.5 mL Acetic acid was added after 1.5 min. The solution was then heated to 100°C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine. Blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Different concentration of dopamine and nor-adrenaline (1 mg/ml) was used as standard [64].

5.8 b. Estimation of Serotonin

The serotonin content was estimated by the OPT method

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 mL aqueous extract, 1.75 mL of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/mL) at different concentration was used as standard [78].

5.9 *In vivo* antioxidant activity

5.9 a. **Estimation of proteins**

Requirements

- Alkaline copper reagent
- Solution A: 2% sodium carbonate in 0.1 N NaOH
- Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate .50 ml of solution   A was mixed with 1 ml of solution B just before use.
- Folin's phenol reagent (commercial reagent, 1:2 dilutions),
- Bovine serum albumin (BSA).

Principle

This method is a combination of both Folin-ciocalteau and biuret reaction which involves two steps

Step 1: Protein binds with copper in alkaline medium and reduces it to Cu++.

Step 2: The cu++ formed catalyzes the oxidation reaction of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum ,which leads to the formation of blue color and absorbance was measured at 640 nm.

Procedure:

To 0.1 ml of the homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand at the room temperature for 10 minutes. To this 0.5 ml of folin'reagent was added. After 20 minutes, the color developed was measured at 640 nm. The level of protein present was expressed as mg/g/ tissue or mg/dl [79].

5.9.b Catalase: [CAT]

Principle:

$$2 H_2O_2 \xrightarrow{\text{CAT}} H_2O + O_2$$

In presence of CAT, H_2O_2 shows a continual decrease in absorbance when measured in UV range. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240nm (E240=0.00394±0.0002 litres m mol^{-1}mm^{-1}). The difference in absorbance (ΔA 240) per unit time is a measure of the CAT activity.

. **Reagents:**

1. Preparation of Phosphate Buffer (PB):

   • KH2PO4 (Potassium dihydrogen phosphate): 1.703 g made upto 250 ml
   • Na2HPO4 (Disodium hydrogen phosphate): 1.773 g made upto 250 ml

100mL of KH2PO4 solution and 150 ml of Na2HPO4 was mixed & pH was adjusted to7.

   2. Preparation of PB- H2O2 solution: 50 ml of PB + 500 µl H2O2

Critical step:

The absorbance of PB- H2O2 solution was checked and it should be between 0.3-0.5. If the absorbance lies below this range, then H2O2 should be added to increase absorbance and if the absorbance lies above this range, PB was added to decrease absorbance. Time course between 0 & 60 second was selected. Auto zero was selected at 240 nm with PB.

Procedure [80]

- 3ml of H2O2 PB solution was added to 50µl of tissue homogenate.
- The above solution was kept in cuvette and absorbance was taken at 240nm.

5.9. c Superoxide Dismutase [SOD]

Principle:

The activity of SOD was determined by the method based upon the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH. Inhibition of the chromogen formation by superoxide dismutase is linear with increase in enzyme concentration [65]:

Reagents:

1. Sodium carbonate buffer 0.1 M (pH 10.2): 1.05g of Na2CO3 in 100 mL of distilled water.
2. Adrenaline (bitartarate) (final concentration- 250 µM)

Procedure:

- 1.85ml of sodium carbonate buffer was taken in the cuvette and 50µl of tissue homogenate was added followed by 100µl of Adr directly to the cuvette kept in the UV

cuvette holder.

- Absorbance was read at 295 nm. The SOD activity (U/mg of protein) was calculated using the standard plot. (Photometric method) [53].

5.10 Determination of lipid peroxidation

Requirements

- Thiobarbituric acid 0.37 %
- 0.25 N HCl
- 15%TCA

Principle

This assay is based on the reduction of thiobarbituric acid with malonyl dialdehyde which is a formed as a result of polyunsaturated fatty acid oxidation. This reaction leads to the formation of pink colored TBA-MDA complex which is measured at 532 nm.

Procedure:

To 0.1 ml of sample, 2 ml of TBA-TCA-HCl reagent (ratio of 1:1) was added mixed and kept in a water bath for 15 minutes. Afterward the solution was cooled and supernatant was removed and absorbance was measured at 535 nm against reference blank. The level of lipid peroxides was given as nm moles of MDA formed/mg protein. [81].

6. Results

6.1 Standardization of ayurvedic formulation saraswatha ghrita

Table No: 2 Organoleptic characteristic of various sensory characters like colour, odour, taste etc,was carefully noted down in table.the SG studied by organoleptic and morphological character like Rupa (colour), Rasa(taste), Gandha(odour), sparsha (touch), and so on.

1.	Colour	Muddy -Green
2.	Odour	Ghee like
3.	Taste	Low sweet
4.	Touch	Sticky
5.	Appearance	Viscous, semi solid

Table No: 3 Evaluation of physicochemical parameters of Ayurvedic Formulation Saraswatha Ghrita.

SL. NO.	PARAMETER	RESULT n =3 % w/w
1	Specific gravity	0.9884
2	Acid value	5.331
3	Saponification value	112.63
4	Iodine value	46.72
5	Unsaponifiable matter	1.003
6	Peroxide value	0.18
7	Rancidity	Fat is not oxidized

6.2 Screening of anti amnesic activity of SG by Morris water maze

The data obtained on the effect of treatment with various doses of SG on learning and special memory are shown in Fig. no: 8 and Table no: 4. The data reveal that induction with scopolamine significantly increases the escape latency time to 31.667 ± 1.174. Treatment with various doses of SG significantly decreases escape latency time (8.500 ± 0.619-low dose, 6.333 ± 0.558-medium dose and 6.167 ± 0.601-high dose, resp.,) Moreover, the highest dose of SG shows the escape latency time (6.167 ± 0.601) as that of Donepezil treated group (6.167 ± 0.307).

Table No: 4 Effect of various doses of SG (100 mg/kg, 200 mg/kg, 400 mg/kg) on leaning and special memory. Each value represents the mean $\pm$ SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

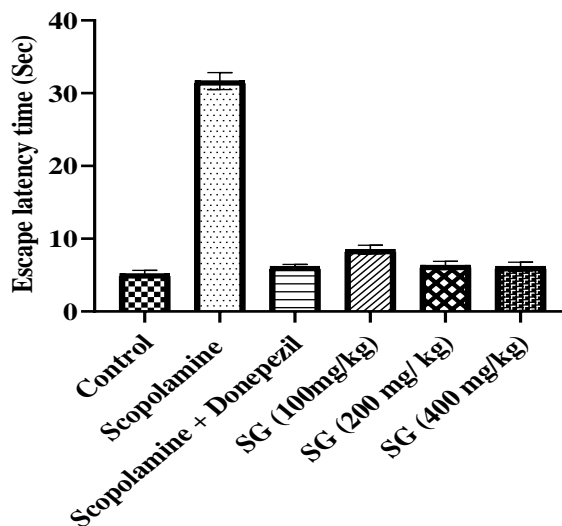| GROUPS	ESCAPE LATENCY TIME in SEC
Control	5.167 ± 0.477 ****
Scopolamine	31.667 ± 1.174
Scopolamine ± Donepezil	6.167 ± 0.307 ****
Low Dose-SG (100 Mg/Kg)	8.500 ± 0.619 ***
Medium Dose-SG(200 Mg/Kg)	6.333 ± 0.558 ****
High Dose-SG (400 Mg/Kg)	6.167 ± 0.601****

Fig.No: 8 Effect of various doses of SG (100 mg/kg, 200 mg/kg, 400 mg/kg) on leaning and special memory on Morris water maze. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group

6.3 Screening of anti amnesic activity of SG by open Field

The data obtained on the effect of treatment with various doses of SG on exploratory behavior are shown in Fig. no: 9a and 9b and Table no: 5. The data reveal that induction with scopolamine significantly decreases the number of rearing and grooming to12.667 ± 1.022 and 6.833 ± 0.833 resp.,. Treatment with various doses of SGsignificantly increases number of rearing and grooming (low dosec19.833 ± 0.910 and 10.500 ± 0.619, medium dose 19.167 ± 0.601 and high dose 11.167 ± 0.477 and 18.667 ± 0.667 and 12.333 ± 0.494) resp.,. Moreover, the highest dose of SG (12.333 ± 0.494 and 18.667 ± 0.667) shows a nearly equal number of rearing and grooming as that of Donepezil treated group (21.500 ±1.232 and 13.167 ± 0.703) resp.,.
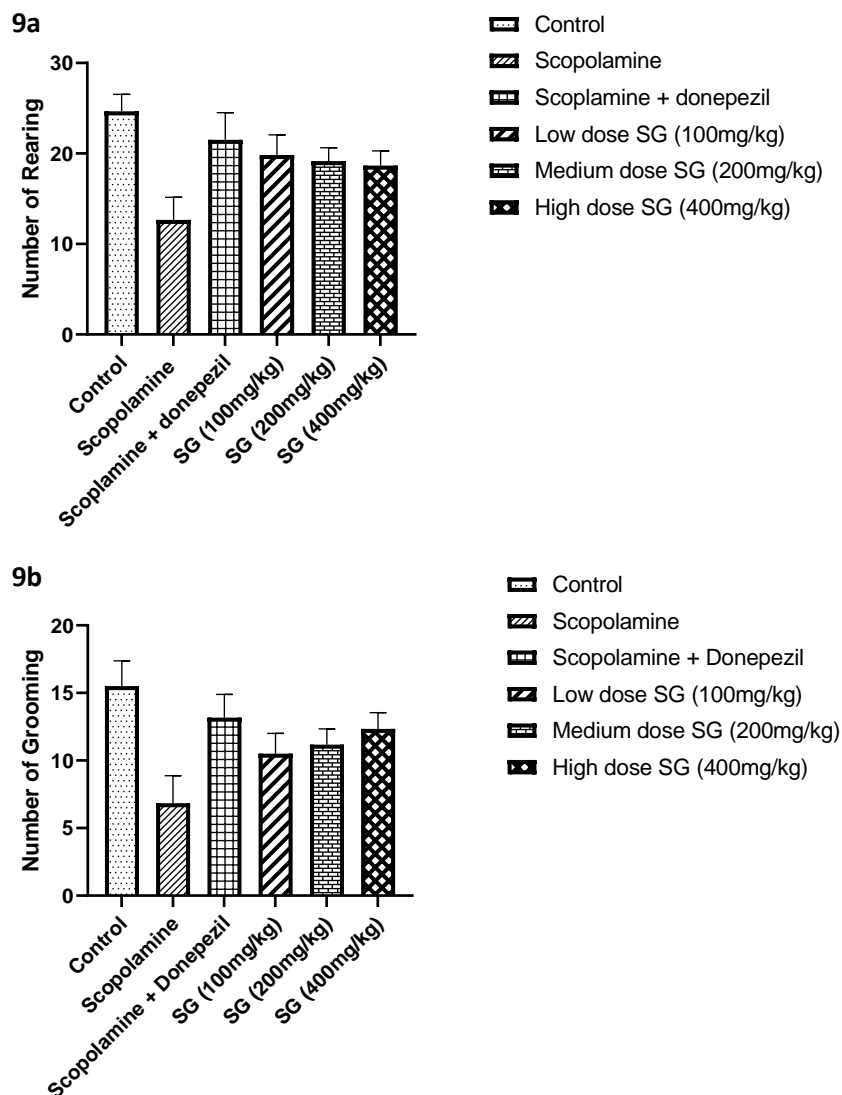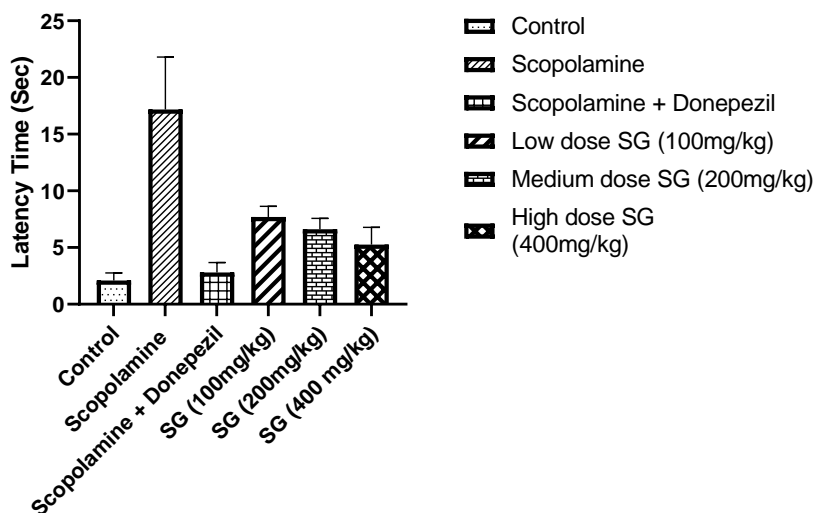
Table No: 5 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on exploratory behavior on open field apparatus. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

GROUP	REARING	GROOMING

Control	24.667 ± 0.760 **	15.500 ±0.764 **
Scopolamine	12.667 ± 1.022	6.833 ± 0.833
Scopolamine + Donepezil	21.500 ±1.232 **	13.167 ± 0.703 **
Low Dose-SG (100 Mg/Kg)	19.833 ± 0.910 *	10.500 ± 0.619 ***
Medium Dose-SG(200 Mg/Kg)	19.167 ± 0.601 **	11.167 ± 0.477 *
High Dose-SG (400 Mg/Kg)	18.667 ± 0.667 **	12.333 ± 0.494 **

.

9a

9b

Fig. No: 9a, 9b Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on exploratory behavior on open field apparatus. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6.4 Screening of anti amnesic activity of SG by passive avoidance test

The data obtained on the effect of treatment with various doses of saraswatha Grita on learning and special memory are shown in Fig. no: 10a and 10b and Table no: 6. The data reveal that induction with scopolamine significantly increases the no. of attempts and latency time to 10.833 ± 0.792 and 17.167 ± 1.893 resp.,. Treatment with various doses of SG significantly decreases the no. of attempts and latency time (3.167 ± 0.307 and $7.700 \pm$

0.383, 2.667 ± 0.211 and 6.617 ± 0.387 and 2.333 ± 0.211 5.267 ± 0.616) resp.,. Moreover, the highest dose of SG (2.333 ± 0.211 and 5.267 ± 0.616) shows a nearly equal the no. of attempts and latency time as that of Donepezil treated group (2.333 ± 0.211 and 2.8 ± 0.358) respectively.

Table No: 6 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on learning and special memoryon passive avoidance apparatus. Each value represents the mean ± SEM (n=6). * $p < 0.1$, ** $p < 0.01$ and *** $p < 0.001$ in comparison with scopolamine treated group.

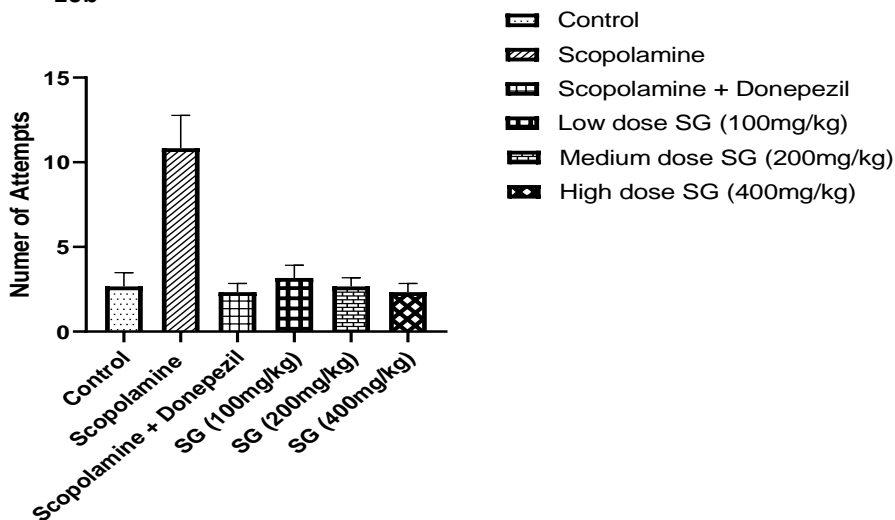| GROUPS	NO. OF ATTEMPTS	LATENCY TIME (SEC)
Control	2.667 ± 0.33 ***	2.100 ± 0.271 **
Scopolamine	10.833 ± 0.792	17.167 ± 1.893
Scopolamine + Donepezil	2.333 ± 0.211 ***	2.8.. ± 0.358 **
Low Dose-SG (100 Mg/Kg)	3.167 ± 0.307 ***	7.700 ± 0.383 *
Medium Dose-SG(200 Mg/Kg)	2.667 ± 0.211 ***	6.617 ± 0.387 *
High Dose-SG (400 Mg/Kg)	2.333 ± 0.211 **	5.267 ± 0.616 **

10a

10b

Fig. No: 10a, 10b : Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on learning and special memoryon passive avoidance apparatus. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6.5 Effect of SG on Acetylcholine esterase level on rat brain

The data obtained on the effect of treatment with various doses of saraswatha Grita on acetyl cholinesterase levels in the brain are shown in Fig. no: 11and Table no:7. The data reveal that induction with scopolamine significantly increases the level of AChE in the brain to 1.142 ± 0.049. Treatment with various doses of SG significantly decreases the level of AChE in the brain (low dose 0.903 ± 0.021, medium dose 0.712 ± 0.023 and high dose 0.611 ± 0.027) resp.,. Moreover, the highest dose of SG (0.611 ± 0.027) shows a nearly equal level of AChE as that of Donepezil treated group (0.571 ± 0.024).

Table No: 7 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on learning and special memoryon passive avoidance apparatus. Each value represents the mean $\pm$ SEM (n=6). * $p<0.1$, ** $p<0.01$ and *** $p<0.001$ in comparison with scopolamine treated group.

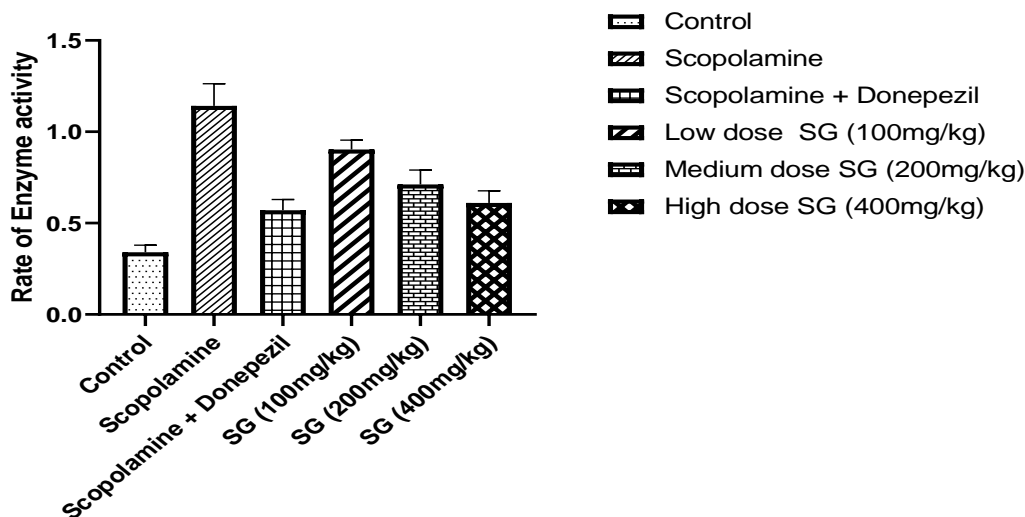| GROUPS	AChE
Control	0.341 ± 0.016 ****
Scopolamine	1.142 ± 0.049
Scopolamine Donepezil	0.571 ± 0.024 ****
Low Dose-SG (100 Mg/Kg)	0.903 ± 0.021 *
Medium Dose-SG(200 Mg/Kg)	0.712 ± 0.023 ***
High Dose-SG (400 Mg/Kg)	0.611 ± 0.027 ****

Fig. No: 11 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) acetyl cholinesterase levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6.6 Estimation of neurotransmitters

6.6 a. Effect of SG on dopamine on amnesic rat brain

The data obtained on the effect of treatment with various doses of saraswatha Grita on dopamine levels in the brain are shown in Fig. no: 12 and Table no: 8. The data reveal that induction with scopolamine significantly decreases the level of dopamine in the brain to 18.485 ± 0.264. Treatment with various doses of SG significantly increases the level of dopamine in the brain (low dose 20.166 ± 0.295, medium dose 20.529 ± 0.176 and high dose 22.101 ± 0.094) respectively. Moreover, the highest dose of SG (22.101 ± 0.094) shows a slightly higher level of dopamine than that of Donepezil treated group (20.590 ± 0.212).

6. 6 b. Effect of SG on level of serotonin on amnesic rat brain

The data obtained on the effect of treatment with various doses of saraswatha Grita on serotonin levels in the brain are shown in Fig. no: 13 and Table no: 8. The data reveal that induction with scopolamine significantly decreases the level of serotonin in the brain (0.885 ± 0.021). Treatment with various doses of SGsignificantly increases (P< 0.1) the level of dopamine in the brain (1.409 ± 0.115, 1.280 ± 0.064 and 1.654 ± 0.159) respectively.

Moreover, the highest dose of SGshows a nearly equal significant effect as that of Donepezil treated group (1.732 ± 0.61).

Table No: 8 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on dopamine and serotonin levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group**.**

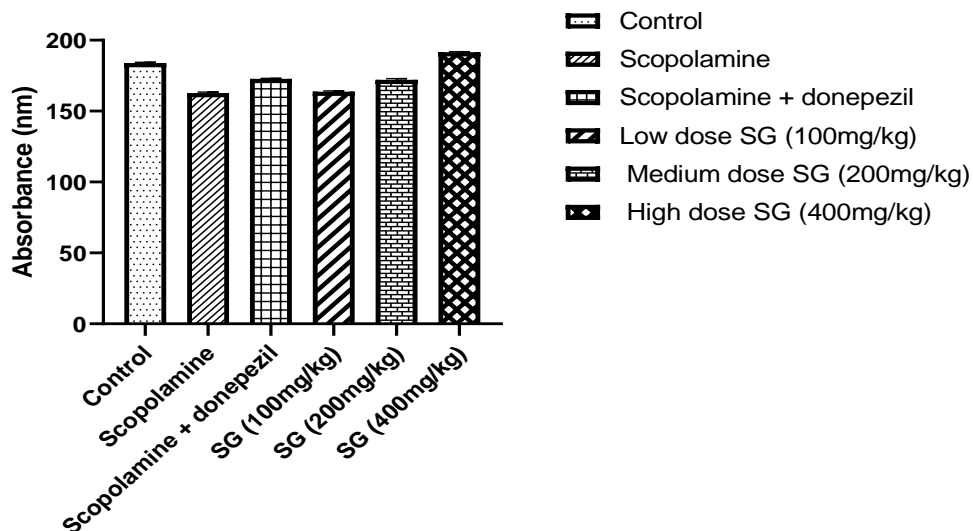| GROUPS	DOPAMINE	SEROTONIN
Control	21.641± 0.385 ***	1.706±0.100 **
Scopolamnie	18.485 ± 0.264	0.885 ± 0.021
Scopolamine ± Donepezil	20.590 ± 0.212 **	1.732 ± 0.61 ***
Low Dose-SG (100 Mg/Kg)	20.166 ±0.295 ns	1.409 ± 0.115 *
Medium Dose-SG(200 Mg/Kg)	20.529 ±0.176 **	1.280 ± 0.064 *
High Dose-SG (400 Mg/Kg)	22.101 ± 0.094 ****	1.654 ± 0.159 *

Fig. No: 12 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on dopamine levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.
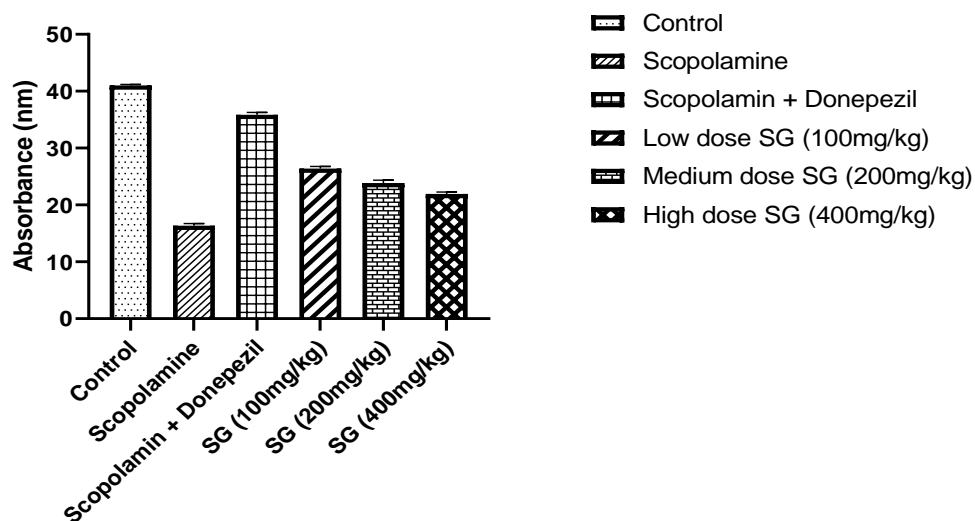

Fig. No: 13 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on serotonin levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6. **7 Estimation of total Proteins:-**

The data obtained on the effect of treatment with various doses of saraswatha Grita on level of total protein are shown in Fig. no: 14 and Table no: 9. The data reveal that induction with scopolamine significantly decreases the level of total protein to 1.989±0.006. Treatment with various doses of SG significantly increases the level of dopamine in the brain (low dose 2.983±0.003, medium dose 3.667±0.070 and high dose 4.079±0.052) respectively. Moreover, the highest dose of SG (4.079±0.052) shows a nearly equal level of total protein as that of Donepezil treated group (4.960±0.012).

Table No: 9 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on total protein levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

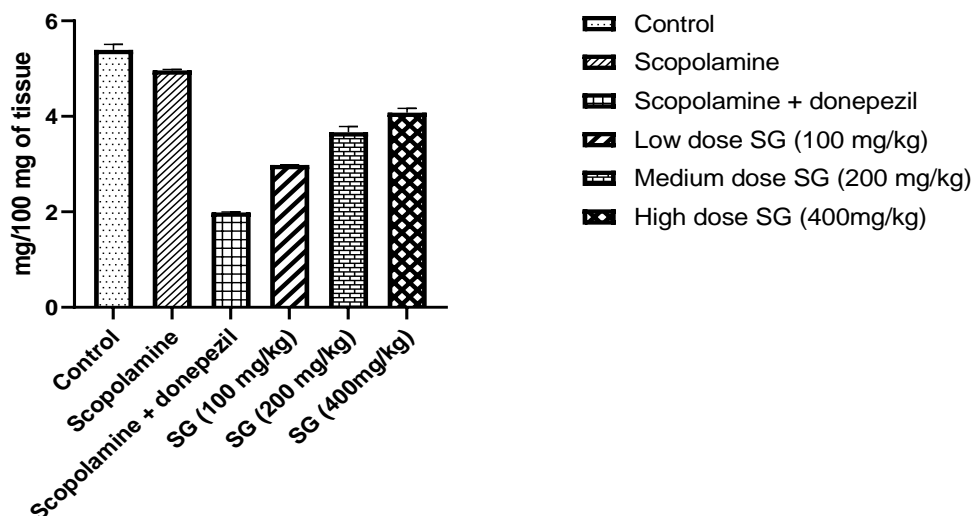| GROUPS	LEVEL OF TOTAL PROTEIN (mg/100mg of tissue)
Control	$5.390 \pm 0.067^{***}$
Scopolamine	1.989 ± 0.006
Scopolamine ± Donepezil	$4.960 \pm 0.012^{***}$
Low Dose-SG (100 Mg/Kg)	$2.983 \pm 0.003^{***}$
Medium Dose-SG(200 Mg/Kg)	$3.667 \pm 0.070^{***}$
High Dose-SG (400 Mg/Kg)	$4.079 \pm 0.052^{***}$

Fig. No: 14 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on total protein levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6. 8 Estimation of various anti oxidant enzyme levels

6. 8 a. Effect of SG on level of LPO in amnesic rat brain

The data obtained on the effect of treatment with various doses of saraswatha Grita on level of various anti oxidant enzymes like LPO in the brain are shown in Fig. no: 15 and Table no:10. The data reveal that induction with scopolamine significantly increases the level of LPO to 0.141 ± 0.002. Treatment with various doses of SG significantly decreases the level of LPO in the brain (low dose 0.126 ± 0.001, medium dose 0.128 ± 0.011 and high dose 0.106 ± 0.001) resp.,. Moreover, the highest dose (0.106 ± 0.001) of SG shows more level of LPO than that of Donepezil treated group (0.130 ± 0.001).

6. 8 b. Effect of SG on level of CAT in amnesic rat brain

The data obtained on the effect of treatment with various doses of saraswatha Grita on level of various anti oxidant enzymes like CAT in the brain are shown in Fig. no: 16 and Table no: 10. The data reveal that induction with scopolamine significantly decreases the level of CAT to 5.552 ± 0.037. Treatment with various doses of SG significantly increases the level of CAT in the brain low dose (6.900 ± 0.074, medium dose 7.114 ± 0.075 and high dose

8.020 ± 0.051) resp.,. Moreover, the highest dose of SG (8.020 ± 0.051) shows a nearly equal level of CAT as that of Donepezil treated group (1.082 ± 0.026).

6. **8 c**. **Effect of SGon level of SOD in amnesic rat brain**

The data obtained on the effect of treatment with various doses of saraswatha Grita on level of various anti oxidant enzymes like SOD in the brain are shown in Fig. no: 17 and Table no: 10. The data reveal that induction with scopolamine significantly increases the level of SOD to 2.655 ± 0.069. Treatment with various doses of SG significantly decreases the level of SOD in the brain (low dose 1.561 ± 0.020, medium dose 1.264 ± 0.025 and high dose 0.656 ± 0.027) respectively. Moreover, the highest dose of SG (0.656 ± 0.027) shows more level of SOD than that of Donepezil treated group (1.082 ± 0.026).

Table No: 10 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on various anti oxidant enzymes like LPD, SOD and CAT levels. Each value represents the mean ± SEM (n=6). * $p<0.1$, ** $p<0.01$ and *** $p<0.001$ in comparison with scopolamine treated group.

| GROUPS	LPO (No. Mol of MDA/mg Protein)	SOD (U/mg of brain tissue)	CATALASE (U/mg of brain tissue)
Control	0.104 ± 0.001 ****	0.530 ±0.007 **	11.173 ±0.091 **
Scopolamine	0.141 ± 0.002	2.655 ±0.069	5.552 ±0.037
Scopolamine ± Donepezil	0.130 ± 0.001 **	1.082 ± 0.026 **	8.116 ± 0.089 **
Low Dose-SG (100 Mg/Kg)	0.126 ± 0.001 **	1.561 ± 0.020 *	6.900 ± 0.074 *
Medium Dose-SG (200 mg/Kg)	0.128 ± 0.011 ns	1.264 ± 0.025 *	7.114 ± 0.075 **
High Dose-SG (400 Mg/Kg)	0.106 ± 0.001 ****	0.656 ± 0.027 **	8.020 ± 0.051 **
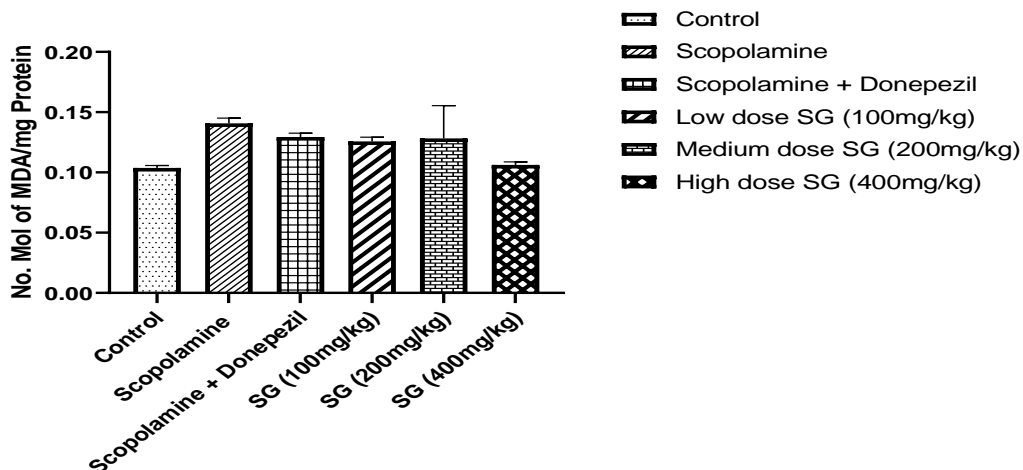
Fig. No: 15 Effect of various doses of SG (100 mg/kg, 200 mg/kg, 400 mg/kg) on various anti oxidant enzymes like LPO levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.
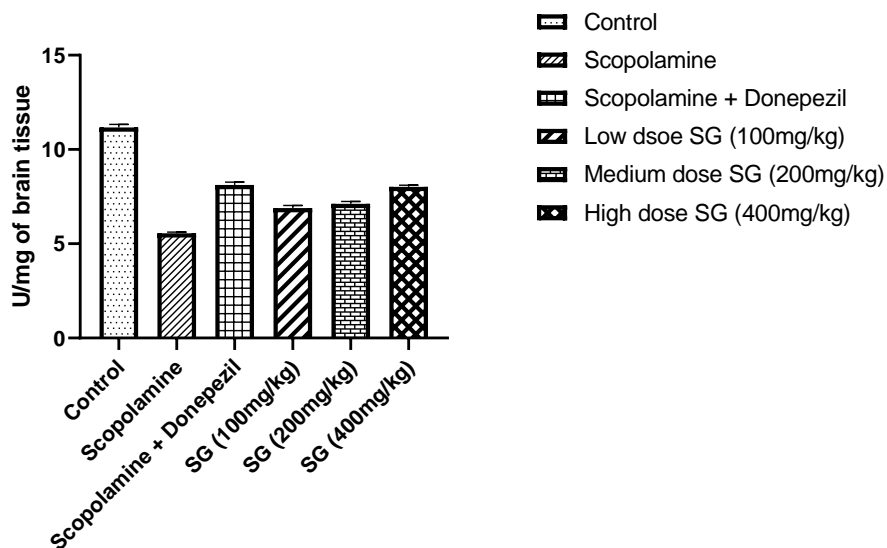

Fig. No: 16 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on various anti oxidant enzymes like CAT levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.
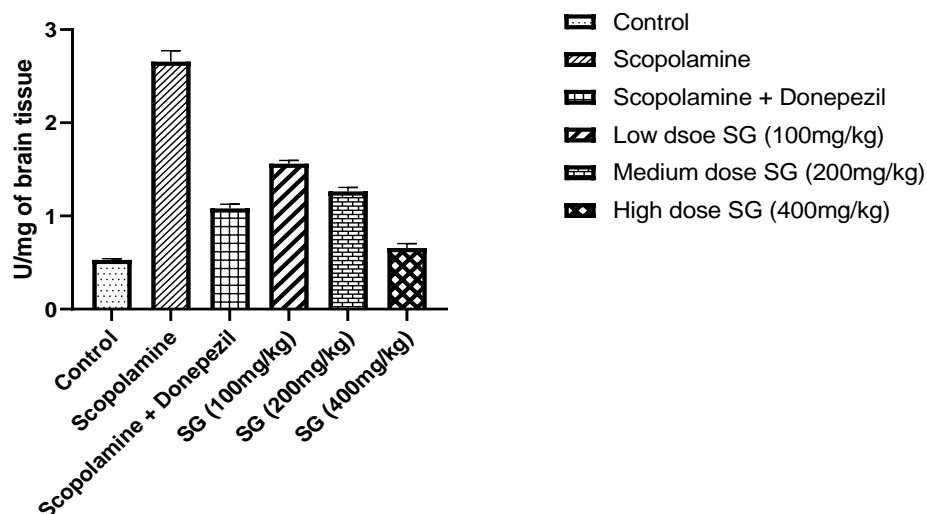
Fig. no: 17 Effect of various doses of SG(100 mg/kg, 200 mg/kg, 400 mg/kg) on various anti oxidant enzymes like SOD levels. Each value represents the mean ± SEM (n=6). * p<0.1, ** p<0.01 and *** p<0.001 in comparison with scopolamine treated group.

6. 9 Histopathology

Effect of SG brain tissues of various groups of animals

The results obtained from the histopathological analysis of the effect of treatment with various doses of saraswatha Grita on scopolamine induced rat brain are shown in Fig. no: 18. **A**. The histopathology of rat from the control group which shows normal astrocytes and glial cells. **B.** Brain tissue of scopolamine induced rat which shows reactive gliosis, extensive areas of necrosis and hemorrhage. Collections of inflammatory cells are also presnt. **C**. Brain tissue of standard group which is treated with Donepezil. It shows tissue with normal astrocytes and glial cells. There is minimal necrosis. **D**. Brain tissue of test group which treated with lowest dose of SG (SG) (100 mg/kg). It shows normal astrocytes and glial cells with very few small necrotic areas ansd inflammatory cell collections are seen. **E**. Brain tissue of test group with medium dose of SG (200 mg/kg). It also shows normal astrocytes and glial cell with small necrotic area tan the lower dose treaded group. **F.**

It is the final group treated with highest dose of SG (400 mg/kg). It shows normal astrocytes and glial cells as normal tissue. There is no necrosis at all.
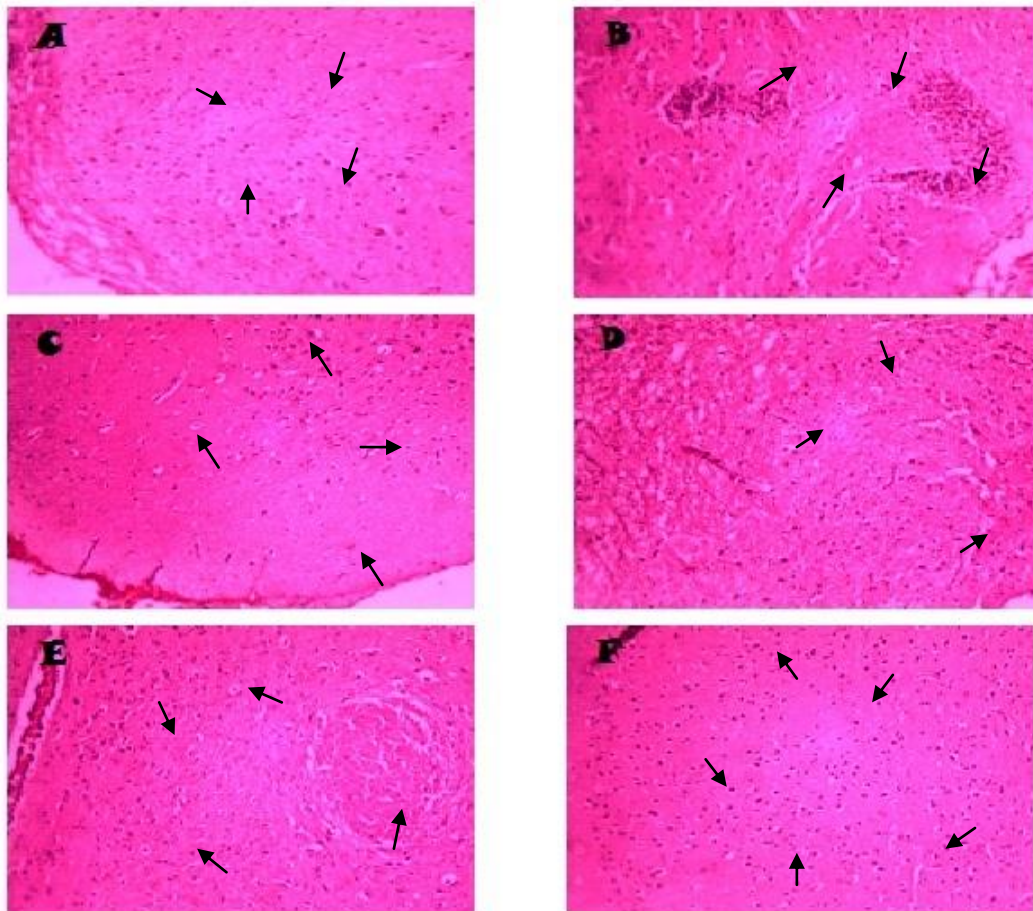

Fig. No: 18 Histopathological analysis of effect of various doses of SG (Low dose 100 mg/kg, Medium dose 200 mg/kg, High dose 400 mg/kg) on rat brain tissue after induction with scopolamine.

7. Discussion

This study aims to evaluate the Anti-Amnesic activity of Ayurvedic formulation SG on scopolamine induced animal model by analyzing behavioral changes, estimation of catecholamine levels, various endogenous antioxidant enzymes levels and histopathological evaluation. Today 25% individuals around the world suffer from mental/neurological disorders in their life time, it is important to find out the more effective drugs for their treatment [82]. Since the use of traditional medicines for the neurological diseases have been concerned, the scientific validation of various medicinal plants supports their traditional uses and have led to development of novel drugs [83,84]. Memory includes storing, retaining, and later recalling information. Memory consolidation and recall are dependent upon complex mnemonic mechanisms, which vary based on the type of memory representation [1]. One of the most promising approaches to treat this disease is enhancing the acetylcholine level in the brain using acetylcholinesterase (AChE) inhibitors [85]. Tacrine, donepezil, rivastigmine, and galanthamine are the major drugs which have been approved by the Food and Drug Administration in the United States used to treat this disease [86]. But, nausea, vomiting, diarrhea, weight loss, decreased appetite, muscle weakness, muscle cramps, fatigue, dizziness and headache are the common side effects obtained for these drugs [87]. Therefore, the search for new AChE inhibitors especially from plants with less side effects has great interest [88]. So that, in the current scenario the present study has a very good relevance which explains the effects of various plant constituents to treat the amnesic conditions.

There is evidence both from animal and human studies indicating that learning and memory can be modified by drugs which affect central cholinergic functions. For instance, scopolamine, a muscarinic receptor blocking drug, has been shown to impair memory [26]. Scopolamine induced memory impairment is one of the most widely used model because complex surgical procedures are not required [28]. Administration of scopolamine produces deficits on tests of visual recognition memory, visuospatial praxis, verbal recall, visuospatial recall, psychomotor speed and visuoperceptual function [27].

In Ayurveda medicinal plants and formulations are routinely used to cure neurodegenerative diseases mainly Alzheimer's disease and its associated symptoms. In this context, Saraswatha Ghritha (SG), an Ayurvedic formulation which contains 7 major ingredients, *Terminalia*

chebula , *Zingiber officinale*, *Acorus calamus*, *Piper longum, Piper nigram, Cyclea peltata and Moringa oleifera* which already reported for free radical scavenging activity, neuro protective and anti inflammatory activity is one of the promising formulation for treating amnesia mainly by altering the levels of Ach and by reducing neuroinflammation has been explored in the present study.

On bases of initial observations, it is semi solid muddy green preparation made with decoction of 7 plant extractive juices in ghee, slightly sweetish, viscous, sticky and without sedimentation. Organoleptic evaluation of SG was performed, the results were satisfactory. The specific gravity of SG was 0.9884g/cm^3 showing the sample was not too dense. The saponification values was found to be 112% w/v, it gave an idea for molecular weight of an oil/fat. So this indicates SG contains more lower molecular saturated fatty acids, which help in better bioavailiablty. Also it contains poly unsaturated fatty acids (PUFA) ie, DHA- Omega 3 long chained PUFA in fair amounts. Intake of PUFA in moderate levels is found to improve levels of acetyl choline thus reducing chances of dementia [89]. Higher the iodine value >55 w/v means the formulation is more susceptible to oxidation, free radical production, polymerization and rancidity. So iodine value intern reflects a products stability and shelf life. Here value is 46 so the product is stable. If acid values are high, then chances of photo oxidation and rancidity is more, in SG acid value is in permissible range 5.33. The rancidity test confirms there is no oxidation of fat, unrancid formulation. Fingerprint identity of SG by its unique R_f values was detected by HPTLC. So based on standardization tests the quality and identity of the product was confirmed for further animal tests.

Based on protocol the various doses of SG were administrated, followed by single dose induction with scopolamine (2mg/kg ip). The cognitive impairment was confirmed by various behavioral studies. Morris water maze results were promising. As it is a standard apparatus for evaluation of learning and special memory, in which the escape latency time is noted. The escape latency time is an indication of cognitive impairment, which gives a clear picture of loss of memory. Control group possess 5.167 ± 0.477 range of learning and special memory. The group of animal induced with scopolamine significantly increases the escape latency time at a range of 31.667 ± 1.174. Treatment with various doses of SG significantly decreases escape latency time (8.500 ± 0.619-low dose, 6.333 ± 0.558-medium   dose and 6.167 ± 0.601-high dose, resp.,) Moreover, the

highest dose of SG shows the escape latency time (6.167 ± 0.601) as that of Donepezil treated group (6.167 ± 0.307). So it is

Passive avoidance test results showed a marked difference in latency time and number of attempts between the untreated and treated groups. So this is a fear-aggravated **test** for evaluation of learning and memory in rodent models of CNS disorders. This confirms the effect of SG on the cognitive impairment caused by scopolamine. Here also the latency time and number of attempt were evaluated. The learning and special memory for control group were 2.667 ± 0.33 and 2.100 ± 0.271 resp., In the scopolamine treated group, there was an increase in the latency time and no. of attempts at a range of 10.833 ± 0.792 and 17.167 ± 1.893 resp., while the SG treated group shows a decreased latency time in a rage of 3.167 ± 0.307 and 7.700 ± 0.383, 2.667 ± 0.211 and 6.617 ± 0.387 and 2.333 ± 0.211 5.267 ± 0.616 resp.,

In open field test the control group showed rearing and grooming in a range of 24.667 ± 0.760 and 15.500 ±0.764 resp., which is a significant result. But the scopolamine induced group of animals has shown a decreased no. of rearing and grooming which is 12.667 ± 1.022 6.833 ± 0.833 resp., while the standard group treated with donepezil (21.500 ±1.232 and 13.167 ± 0.703) and SG treated group have shown a significant increase in the no. of rearing and grooming like 19.833 ± 0.910, 19.167 ± 0.601, 18.667 ± 0.667, 10.500 ± 0.619, 11.167 ± 0.477 and 12.333 ± 0.494 resp., So, open field test is known for often used as a test for anxiety, exploration, and locomotion [90].

The scopolamine treated group showed an increased level of AChE in the brain tisuue at a range of 1.142 ± 0.049 while the SG treated groups showed a decreasing level of AChE in the brain tissue in a dose dependant manner. In the present study, after induction with scopolamine , it non - selectively blocks the binding sites of acetylcholine (ACh) which is a muscarinic receptors in the cerebral cortex and consequences in disproportionate release of Ach by attenuating the level of AChE in the brain which destroys the hippocampus nerves and causes impairment in learning and memory. So, the estimation of AChE level in the brain gives a very good result in evaluating the memory imparment.

Dopamine in the prefrontal cortex is known to play an important **role** in working **memory** by increasing the activity of brain circuits relevant to a task and suppressing circuits that distract from that task. The treatment with scopolamine decreased the level of dopamine in the brain tissue which indicated the range of memory deficit. At the same time, the treatment with SG significantly increased the level of dopamine in the brain tissue as 20.166 ±0.295, 20.529 ±0.176 and 22.101 ± 0.094 with increasing dose.

In a similar way the serotonergic system plays a significant role in learning and memory, in particular by interacting with the cholinergic, glutamatergic, dopaminergic or GABAergic systems. Its action is mediated via specific receptors located in crucial brain structures involved in these functions, primarily the septohippocampal complex and the nucleus basalis magnocellularis (NBM)-frontal cortex [91]. So evaluating the levels of serotonin are important. The scopolamine induced group showed a decreased level of serotonin in the brain tissue while the SG treated group recovered the memory impairment by increasing the level of serotonin. The SG showed a highly significant result compared to the standard drug treated group and scopolamine induced groups

Accumulation of oxidative damage and reduction of antioxidant defense system play a key role in memory [92].Oxidative damage in the brain may lead to cognitive impairments [93]. After treatment with SG the antioxidant enzyme levels in brain regained to normal values. Example, SOD level was increased in scopolamine induced group. But the donepezil treated group has shown a lower level of SOD like 0.530 ± 0.007. While the various dosed SG showed significant decrease like 1.264 ± 0.025, 1.561 ± 0.020 and 0.656 ± 0.027 in the SOD level. Like wise, in catalase the scopolamine induced groups showed a decrease (5.552 ± 0.037 when compared with the standard group and SG treated group. When comparing the standard group (11.173 ± 0.091)and SG treated groups (6.900 ± 0.074, 7.114 ± 0.075 and 8.020 ± 0.051), the SG treated groups have shown a very good significance. In SG treated groups the values of LOP were also promising compared with the standard treatment. Histopathology results confirmed its effect on neuroinflamation. Necrosis, inflammatory cells and hemorrhage were clearly observed in scopalmine induced brain tissue. But SG treated group brain tissues were perfectly normal. So this give a clear confirmation that SG can be used for treatment of cognitive impairment disorders.

8. Conclusion

From the present study, it can be considered that the Ayurvedic formulation Saraswatha Ghrita exhibited significant anti-amnesic activity in scopolamine induced rat model. All the Parameters of formulation treated group have shown better results when compared with scopolamine induced -group. Mechanism behind the anti amnesic activity of Saraswatha Ghrita is by its acetyl cholinesterase inhibition, free radical scavenging and neuro protective properties.

9. **Bibliography**

1. Raymond P. Kesnera, Naomi J. Goodrich-Hunsaker. Developing an animal model of human amnesia: The role of the hippocampus. Neuropsychologia. 48; 2010: 2290–2302

2. Anna – Katharine Brem, Kathy Ran, and Alvaro Pascual leone. Learning and Memory. Hand book of Clinical Neurology. 116; 2013

3. Hugo J. Spiers, Eleanor A. Maguire and Neil Burgess. Hippocampal Amnesia. Neurocase (oxford university press) (2001) ; vol. 7: 357-358

4. Renente Rondina II and Jennifer D. Ryan. Evidence From Amnesia and Ageing Inform The Organization of Space and Time in Hippocampal Relational Representations. Current Opinion in Behavioral Sciences. 2017, 17: 77-83

5. Daniel Tranel. Higher Brain Functions. Neuroscience in Medicine, 2nd edition; 2003

6. Christine N. Smitha,b, Jennifer C. Frascinoa,b, Ramona O. Hopkinsc,d, Larry R. Squire. The nature of anterograde and retrograde memory impairment after damage to the medial temporal lobe. Neuropsychologia. 2013

7. Larry r. Squire and stuart m. Zola. Amnesia, memory and brain systems. The Royal Society: 2015

8. Kwon SH, Lee HK, Kim JA, Hong SI, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY, Jang CG. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. European journal of pharmacology. 2010 Dec 15;649 (1-3):210-7

9. Chopin P, Briley M. Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. Psychopharmacology. 1992 Jan 1;106(1):26-30

10. Ortega-de San Luis C, Ryan TJ. United states of amnesia: rescuing memory loss from diverse conditions

11. Lu P, Mamiya T, Lu LL, Mouri A, Zou LB, Nagai T, Hiramatsu M, Ikejima T, Nabeshima T. Silibinin prevents amyloid β peptide-induced memory impairment and oxidative stress in mice. British journal of pharmacology. 2009 Aug;157(7):1270-7

12. Small DH, McLean CA. Alzheimer's disease and the amyloid β protein: what is the role of amyloid?. Journal of neurochemistry. 1999 Aug;73(2):443-9

13. Nunes-Tavares, N.;Santos, L.E.;Stutz, B.;Brito Moreira, J.;Klein, W.L.; Ferreira, S.T.; deMello, F.G. Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid-β peptide oligomers. J. Biol. Chem. 2012, 287, 19377–19385.

14. Götz J, Chen FV, Van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils. Science. 2001 Aug 24;293(5534):1491-5.

15. Desrumaux C, Pisoni A, Meunier J, Deckert V, Athias A, Perrier V, Villard V, Lagrost L, Verdier JM, Maurice T. Increased amyloid-β peptide-induced memory deficits in

phospholipid transfer protein (PLTP) gene knockout mice. Neuropsychopharmacology. 2013 Apr;38(5):817-25.

16. Wiley, P. Isolation and chemistry of streptozotocin. In Streptozotocin: Fundamentals and Therapy; Elsevier North-Holland Biomedical Press: New York, NY, USA, 1981

17. Salkovic-Petrisic M, Osmanovic-Barilar J, Knezovic A, Hoyer S, Mosetter K, Reutter W. Long-term oral galactose treatment prevents cognitive deficits in male Wistar rats treated intracerebroventricularly with streptozotocin. Neuropharmacology. 2014 Feb 1;77:68-80

18. Zhou S, Yu G, Chi L, Zhu J, Zhang W, Zhang Y, Zhang L. Neuroprotective effects of edaravone on cognitive deficit, oxidative stress and tau hyperphosphorylation induced by intracerebroventricular streptozotocin in rats. Neurotoxicology. 2013 Sep 1;38:136-45

19. Dalla Y, Singh N, Jaggi AS, Singh D. Memory restorative role of statins in experimental dementia: an evidence of their cholesterol dependent and independent actions. Pharmacological reports. 2010 Sep 1;62(5):784-96

20. Kim YM, Ji ES, Kim SH, Kim TW, Ko IG, Jin JJ, Kim CJ, Kim TW, Kim DH. Treadmill exercise improves short-term memory by enhancing hippocampal cell proliferation in quinolinic acid-induced Huntington's disease rats. Journal of exercise rehabilitation. 2015 Feb;11(1):5

21. Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 1986 May;321(6066):168-71

22. Orlando LR, Alsdorf SA, Penney Jr JB, Young AB. The role of group I and group II metabotropic glutamate receptors in modulation of striatal NMDA and quinolinic acid toxicity. Experimental neurology. 2001 Jan 1;167(1):196-204

23. Ting KK, Brew BJ, Guillemin GJ. Effect of quinolinic acid on human astrocytes morphology and functions: implications in Alzheimer's disease. Journal of neuroinflammation. 2009 Dec 1;6(1):36

24. Santamaría A, Galván-Arzate S, Lisý V, Ali SF, Duhart HM, Osorio-Rico L, Ríos C, Sut'astný F. Quinolinic acid induces oxidative stress in rat brain synaptosomes. Neuroreport. 2001 Mar 26;12(4):871-4

25. Kumar A, Seghal N, Naidu PS, Padi SS, Goyal R. Colchicines-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type. Pharmacological reports. 2007 May 1;59(3):274

26. Nakagawa Y, Nakamura S, Kaśe Y, Noguchi T, Ishihara T. Colchicine lesions in the rat hippocampus mimic the alterations of several markers in Alzheimer's disease. Brain research. 1987 Apr 7;408(1-2):57-64

27. Evrard PA, Ragusi C, Boschi G, Verbeeck RK, Scherrmann JM. Simultaneous microdialysis in brain and blood of the mouse: extracellular and intracellular brain colchicine disposition. Brain research. 1998 Mar 9;786(1-2):122-7

28. Stone WS, Croul CE, Gold PE. Attenuation of scopolamine-induced amnesia in mice. Psychopharmacology. 1988 Nov 1;96 (3):417-20

29. Flicker C, Serby M, Ferris SH. Scopolamine effects on memory, language, visuospatial praxis and psychomotor speed. Psychopharmacology. 1990 Feb 1;100(2):243-50

30. Kwon SH, Lee HK, Kim JA, Hong SI, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY, Jang CG. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. European journal of pharmacology. 2010 Dec 15;649(1-3):210-7

31. Riedel G, Kang SH, Choi DY, Platt B. Scopolamine-induced deficits in social memory in mice: reversal by donepezil. Behavioural brain research. 2009 Dec 1;204(1):217-25

32. Khakpai F, Nasehi M, Haeri-Rohani A, Eidi A, Zarrindast MR. Scopolamine induced memory impairment; possible involvement of NMDA receptor mechanisms of dorsal hippocampus and/or septum. Behavioural brain research. 2012 May 16;231(1):1-0

33. Akinyemi AJ, Adeniyi PA. Effect of essential oils from ginger (Zingiber officinale) and turmeric (Curcuma longa) rhizomes on some inflammatory biomarkers in cadmium induced neurotoxicity in rats. Journal of toxicology. 2018;2018

34. Shen YC, Juan CW, Lin CS, Chen CC, Chang CL. Neuroprotective effect of terminalia chebula extracts and ellagic acid in pc12 cells. African Journal of Traditional, Complementary and Alternative Medicines. 2017;14(4):22-30.

35. Sadeghnia HR, Jamshidi R, Afshari AR, Mollazadeh H, Forouzanfar F, Rakhshandeh H. Terminalia chebula attenuates quinolinate-induced oxidative PC12 and OLN-93 cell death. Multiple sclerosis and related disorders. 2017 May 1;14:60-7.

36. Sutalangka C, Wattanathorn J. Neuroprotective and cognitive-enhancing effects of the combined extract of Cyperus rotundus and Zingiber officinale. BMC complementary and alternative medicine. 2017 Dec;17(1):135

37. Wang B, Zhang Y, Huang J, Dong L, Li T, Fu X. Anti-inflammatory activity and chemical composition of dichloromethane extract from Piper nigrum and P. longum on permanent focal cerebral ischemia injury in rats. Revista Brasileira de Farmacognosia. 2017 Jun;27(3):369-74

38. Ekong MB, Ekpo MM, Akpanyung EO, Nwaokonko DU. Neuroprotective effect of Moringa oleifera leaf extract on aluminium-induced temporal cortical degeneration. Metabolic brain disease. 2017 Oct 1;32(5):1437-47

39. Liu HL, Luo R, Chen XQ, Ba YY, Zheng L, Guo WW, Wu X. Identification and simultaneous quantification of five alkaloids in Piper longum L. by HPLC–ESI-MSn and UFLC–ESI-MS/MS and their application to Piper nigrum L. Food chemistry. 2015 Jun 15;177:191-6

40. Nabavi SF, Braidy N, Gortzi O, Sobarzo-Sanchez E, Daglia M, Skalicka-Woźniak K, Nabavi SM. Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. Brain research bulletin. 2015 Oct 1;119:1-1

41. Giacoppo S, Galuppo M, Montaut S, Iori R, Rollin P, Bramanti P, Mazzon E. An overview on neuroprotective effects of isothiocyanates for the treatment of neurodegenerative diseases. Fitoterapia. 2015 Oct 1;106:12-21

42. Harandi S, Golchin L, Ansari M, Moradi A, Shabani M, Sheibani V. Antiamnesic effects of walnuts consumption on scopolamine-induced memory impairments in rats. Basic and clinical neuroscience. 2015 Apr;6(2):91

43. Dhanasekaran S, Perumal P, Palayan M. In-vitro Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of Ipomoea aquatica Forsk: therapeutic lead for Alzheimer's disease. Journal of Applied Pharmaceutical Science. 2015 Feb;5(02):012-6

44. Ali-Shtayeh MS, Jamous RM, Zaitoun SY, Qasem IB. In-vitro screening of acetylcholinesterase inhibitory activity of extracts from Palestinian indigenous flora in relation to the treatment of Alzheimer's disease. Functional Foods in Health and Disease. 2014 Sep 1;4(9):381-400.

45. Puri A, Srivastava P, Pandey P, Yadav RS, Bhatt PC. Scopolamine induced behavioral and biochemical modifications and protective effect of Celastrus paniculatous and Angelica glauca in rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2014 Jul 1;4(3):158

46. Hannan MA, Kang JY, Mohibbullah M, Hong YK, Lee H, Choi JS, Choi IS, Moon IS. Moringa oleifera with promising neuronal survival and neurite outgrowth promoting potentials. Journal of ethnopharmacology. 2014 Feb 27;152(1):142-50

47. Hritcu L, Noumedem JA, Cioanca O, Hancianu M, Kuete V, Mihasan M. Methanolic extract of Piper nigrum fruits improves memory impairment by decreasing brain oxidative stress in amyloid beta (1–42) rat model of Alzheimer's disease. Cellular and molecular neurobiology. 2014 Apr 1;34(3):437-49

48. Rasyidah T, Suhana S, Nur-Hidayah H, Kaswandi M, Noah R. Evaluation of antioxidant activity of Zingiber officinale (ginger) on formalin-induced testicular toxicity in rats. Journal of Medical and Bioengineering Vol. 2014 Sep;3(3)

49. Kim HJ, Kim J, Kang KS, Lee KT, Yang HO. Neuroprotective effect of chebulagic acid via autophagy induction in SH-SY5Y cells. Biomolecules & therapeutics. 2014 Jul;22(4):275

50. Sutalangka C, Wattanathorn J, Muchimapura S, Thukham-mee W. Moringa oleifera mitigates memory impairment and neurodegeneration in animal model of age-related dementia. Oxidative medicine and cellular longevity. 2013;2013

51. Chang CL, Lin CS. Phytochemical composition, antioxidant activity, and neuroprotective effect of Terminalia chebula Retzius extracts. Evidence-Based Complementary and Alternative Medicine. 2012;2012

52. Joy AE, Shyamjith M, Bhat KS. Acute Effect of Ethanolic Extract of Moringa oleifera on Haloperidol Induced Catalepsy in Mice Models. Drug Invention Today. 2012 Oct 1;4(10)

53. Ghulam moinuddin, mohammed naseeruddin inamdar, Kala s. KulKarni, Chanda KulKarni, Modulation of Hemodynamics, Endogenous Antioxidant Enzymes, and Pathophysiological Changes by Angiotensin-Converting Enzyme Inhibitors in Pressure-Overload Rats, Hellenic J Cardiol 2011; 52: 216-226

54. Chellappan DR, Joseph J, Balasubramanian P. Pharmacognostical, antioxidant and antiulcer screening of Cyclea peltata roots. Revista Brasileira de Farmacognosia. 2011 Dec;21(6):1096-103

55. Muthuraman A, Singh N. Attenuating effect of Acorus calamus extract in chronic constriction injury induced neuropathic pain in rats: an evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC complementary and alternative medicine. 2011 Dec;11(1):24

56. Wattanathorn J, Jittiwat J, Tongun T, Muchimapura S, Ingkaninan K. Zingiber officinale mitigates brain damage and improves memory impairment in focal cerebral ischemic rat. Evidence-Based Complementary and Alternative Medicine. 2011;2011

57. Shanmugam KR, Mallikarjuna K, Kesireddy N, Reddy KS. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. Food and chemical toxicology. 2011 Apr 1;49(4):893-7

58. Vijayan FP, Rani VJ, Vineesh VR, Sudha KS, Michael MM, Padikkala J. Protective effect of Cyclea peltata Lam on cisplatin-induced nephrotoxicity and oxidative damage. Journal of basic and clinical physiology and pharmacology. 2007;18(2):101-14

59. Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC. Neuroprotective effect of Acorus calamus against middle cerebral artery occlusion–induced ischaemia in rat. Human & experimental toxicology. 2006 Apr;25(4):187-94

60. Cho J, Kim YH, Kong JY, Yang CH, Park CG. Protection of cultured rat cortical neurons from excitotoxicity by asarone, a major essential oil component in the rhizomes of Acorus gramineus. Life Sciences. 2002 Jun 21;71(5):591-9

61. Shukla PK, Khanna VK, Ali MM, Maurya RR, Handa SS, Srimal RC. Protective effect of Acorus calamus against acrylamide induced neurotoxicity. Phytotherapy research. 2002 May;16(3):256-60

62. Topic B, Tani E, Tsiakitzis K, Kourounakis PN, Dere E, Hasenöhrl RU, Häcker R, Mattern CM, Huston JP. Enhanced maze performance and reduced oxidative stress by combined extracts of Zingiber officinale and Ginkgo biloba in the aged rat. Neurobiology of aging. 2002 Jan 1;23(1):135-43

63. Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Molecular and cellular biochemistry. 1995 Oct 1;151(2):113-9

64. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochemical pharmacology. 1974 Sep 1;23(17):2437-46

65. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972 May 25;247(10):3170-5

66. Yuh-Chiang Shen, Chi-Wen Juan, Che-San Lin, Chien-Chih Chen and Chia-Lin Chang. Neuroprotective Effect of *Terminalia Chebula* Extracts and Ellagic Acid in PC12 Cells. Afr J Tradit Complement Altern Med.2017; 14 (4): 22-30.

67. Anwesa Bag, Subir Kumar Bhattacharyya, Nishith Kumar Pal,Rabi Ranjan Chattopadhyay. Anti-inflammatory, Anti-lipid Peroxidative, Antioxidant and Membrane Stabilizing Activities of Hydroalcoholic Extract of *Terminalia Chebula* Fruits 2013:17.

68. Ayodele Jacob Akinyemi , Philip Adeyemi Adeniyi. Effect of Essential Oils From Ginger (Zingiber officinale) and *Turmeric (Curcuma longa) Rhizomes* on Some Inflammatory Biomarkers in Cadmium Induced Neurotoxicity in Rats. Journal of Toxicology. 2018:1-7.

69. Jintanaporn wattanathorn, Jinatta Jittiwat, Terdthai Tongun, Supaporn Muchimapura, Kornkanoklng Kanian.*Zingiber Officinale* Mitigates Brain Damage And Improves Memory Impairment In Focal Cerebral Ischemic Rat. Evidence-Based Complementary And Alternative Medicine.2011;1-8.

70. Gunhyuk PARK, Hyo Geun KIM, Mi Sun JU, Sang Keun HA, Yongkon PARK, Sun Yeou KIM, Myung Sook OH1, 2, 6-Shogaol, An Active Compound Of Ginger, Protects Dopaminergic Neurons In Parkinson's Disease Models via Anti-Neuroinflammation. Acta Pharmacologica Sinica. 2013: 1–9.

71. Wei Yang, Yu-Hua Chen, Hao Liu, Hong-Dang Qu.. Neuroprotective Effects Of *Piperine* On The 1-methyl-4-phenyl-1,2,3,6-Tetrahydropyridine-Induced Parkinson's disease Mouse Model.International Journal of Molecular Medicine.2015; 36: 1369-1376.

72. Alyne Oliveira Correia, Abílio Augusto Pimentel Cruz, Arôdo Tenório Ribeiro de Aquino, Joanisson Rubens Gomes Diniz, et al.,Neuroprotective Effects Of *Piperine*, An Alkaloid From The Piper Genus on the Parkinson's Disease Model in Rats. Journal of Neurology and Therapeutics. 2015, 1(1):1-8

73. Meena J, Santhy KS. Evaluation of the Antioxidant Potential of Methanol Extract of *Cyclea Peltata* in DAL Model. The Pharma Innovation Journal 2015; 4(1): 71-75.

74. David Raj Chellappan, Jipnomon Joseph, Parimaladevi Balasubramanian. Pharmacognostical, Antioxidant and Antiulcer screening of <u>*Cyclea peltata* roots</u>. Rev. Bras. Farmacogn. Brazilian Journal of Pharmacognosy. 2011;21(6):1-8.

75. Venkatramaniah .C, Mary Antony Praba.A , Mohammed Ismail.B . Neuroprotective Role of *Acorus Calamus* and Its Active Principle Beta Asarone in Retaining the Memory of Rats using 8 Arm Radial Maze. Int J Anat Res 2016; 4(2):2238-2244.

76. Sindhu P, Chowta MN, Nishith RS, Rai S, Gadgade A, Rajeshwari S. Evaluation of antidepressant activity of Bacopa monnieri in mice. International Journal of Pharmacy. 2014;4(3):144-8.

77. Nimish L. Pathak, Sanjay B, Nayna M. Bhatt and Ritesh G. Patel. Experimental Modeling of Anxiety. Journal of Applied Pharmaceutical Science, 2011; 01 (03): 06-10

78. Pal DK. Determination of brain biogenic amines in Cynodon dactylon Pers. and Cyperus rotundus L. treated mice. International Journal of Pharmacy and Pharmaceutical Sciences. 2009;1(1):190-7

79. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent.. 7, biol. Chem. 193, 265. 1951;275

80. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972 May 25;247(10):3170-5.

81. Aebi H. Catalase. Methods of enzymatic analysis. Academic Press, New York. 1974; 673–77

82. World Health Organization. The World Health Report 2001: Mental health: new understanding, new hope. World Health Organization; 2001

83. Sheng-Ji P. Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharmaceutical biology. 2001 Jan 1;39(sup1):74-9

84. Ullah I, Subhan F, Ayaz M, Shah R, Ali G, Haq IU, Ullah S. Anti-emetic mechanisms of zingiber officinale against cisplatin induced emesis in the pigeon; behavioral and neurochemical correlates. BMC complementary and alternative medicine. 2015 Dec;15(1):34

85. Davis KL, Thal LJ, Gamzu ER, Davis CS, Woolson RF, Gracon SI, Drachman DA, Schneider LS, Whitehouse PJ, Hoover TM, Morris JC. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. New England Journal of Medicine. 1992 Oct 29;327(18):1253-9

86. Di Giovanni S, Borloz A, Urbain A, Marston A, Hostettmann K, Carrupt PA, Reist M. In vitro screening assays to identify natural or synthetic acetylcholinesterase inhibitors: thin layer chromatography versus microplate methods. european journal of pharmaceutical sciences. 2008 Feb 5;33 (2):109-19

87. Whitehouse PJ. Cholinergic therapy in dementia. Acta Neurologica Scandinavica. 1993 Nov;88(S149):42-5

88. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. Journal of Ethnopharmacology. 2003 Dec 1;89(2-3):261-4

89. Nambiar S, Nidhin MM, Kadibagil VR. CRITICAL REVIEW ON MANAGEMENT OF DEMENTIA WITH GHRITA KALPANA

90. Nordquist RE, Meijer E, van der Staay FJ, Arndt SS. Pigs as Model Species to Investigate Effects of Early Life Events on Later Behavioral and Neurological Functions. InAnimal Models for the Study of Human Disease 2017 Jan 1 (pp. 1003-1030). Academic Press

91. Buhot MC, Martin S, Segu L. Role of serotonin in memory impairment. Annals of medicine. 2000 Jan 1;32(3):210-21

92. Haddadi M, Jahromi SR, Sagar BC, Patil RK, Shivanandappa T, Ramesh SR. Brain aging, memory impairment and oxidative stress: a study in Drosophila melanogaster. Behavioural brain research. 2014 Feb 1;259:60

93. Head E. Oxidative damage and cognitive dysfunction: antioxidant treatments to promote healthy brain aging. Neurochemical research. 2009 Apr 1;34(4):670-8

ABSTRACT

Selective damage to a number of brain regions has been associated with amnesia, including a circuit comprising the hippocampus, the diencephalon and the fibers connecting them. One of the most promising approaches to treat amnesia is enhancing the acetylcholine level in the brain using acetyl cholinesterase (AChE) inhibitors. Classical drugs having side effects like nausea, vomiting, diarrhea, weight loss, decreased appetite, muscle weakness, muscle cramps, fatigue, dizziness and headache Therefore, the search for new AChE inhibitors especially from plants with less side effects has great interest. The aim of the present study is to evaluate the anti amnesia activity of ayurvedic formulation Saraswatha ghrita (SG)in scopolamine induced rat model. For induction of amnesia, scopalamine was injected i.p (single dose of 2 mg/kg) in rats .1 hr before induction various doses of SG (100 mg/kg, 200 mg/kg and 400 mg/kg) were administrated (p.0). To find out its Anti amnesic activity various behavioral studies, biochemical estimations, neurotransmitter evaluations and histopathalological studies have been performed. The results were promising like, in water maze the high dose SG treated group (400mg/kg) escape latency time (6.167 ± 0.601sec) was significantly increased compared to scopolamine induced group (31.667 ± 1.174). Treatment with SG results in regains the levels of endogenous antioxidant levels to normal values. In case of acetyl cholinesterase activity SG treated group there was a significantly decrease level of acetyl cholinesterase (0.611 ±0.027) compared with scopolamine induced group (1.142 ±0.049) especially neuroprotective effect of SG was confirmed by histopathology. The inflammatory cells were absent, glial and astrocytes possess proper morphological features in SG treated group. So this study concludes the Anti amnesic activity of saraswatha gritha.

Keywords: Saraswatha Ghrita , anti-amnesic activity and scopolamine