International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 799-829 www.ijprajournal.com ISSN: 2249-7781

Pharmacological in vivo Screening of Poly Herbal Plant Extract on Alzheimer's Disease Using Zebrafish

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Submitted: 25-03-2024 Accepted: 05-04-2024

ABSTRACT
Alzheimer's disease (AD), is a complex, multifactorial, progressive, neurodegenerative disorder characterized by impairment in learning and memory followed by more global cognitive deficits and behavioural disturbances which become progressively more severe. Alzheimer's disease is the most common form of dementia, accounting for 50%-60% of all cases. The neurological hall mall mark of AD includes deposits of amyloid fibrils in senile plaques and presence of abnormal tau protein filaments in the form of neurofibrillary tangles. Hippocampus, limbic system and cortex are the primary areas involved in the pathophysiology of AD. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. Bacopa monnieri is also known as Bramhi ghratam, it is an herb from India.M.Oleifera is a plant native to northern india that can also grow in other tropical &subtropical places, like Asia and Africa. Folk medicine has used the leaves, flowers, seeds, and Roots, of this plant for centuries. Vitis vinifera is the common grape vine is a species of flowering plant, native to the Mediterranean region. The main aim of this study is to enhance the learning memory and neuroprotection of scopolamine induced Alzheimer's in zebra fish with poly herbal plant extract and compare the activity of the. In the present study,the poly herbal plant extract has been used as a test drug to investigate its poly herbal plant extract and a standard drug-Rivastigmine activity potential for treating the diseased animals.
KEYWORDS: Neurodegenerative disorder, Dementia,Hippocampus, Amyloid fibrils, Senile plaques, Limbic system, Scopolamine Cognitive deficits, Neurofibrillary tangles, Neuroprotection.

I. INTRODUCTION

Alzheimer's disease (AD), is a complex, multifactorial, progressive, neurodegenerative disorder characterized by impairment in learning and memory followed by more global cognitive deficits and behavioural disturbances which become progressively more severe. Patients experience irreversible global, progressive impairment of brain function, leading to reduced intellectual ability. Alzheimer's disease is the most common form of dementia, accounting for 50%-60% of all cases. More than 25 million people in the world are currently affected by dementia, most suffering from AD, with around 5 million new cases occurring every year. The number of people with dementia is anticipated to double every 20 years. Ageing, Apolipoprotien E4 variant (associated with an increased risk of Alzheimer's disease presenting at an earlier age). Head injury, Risk factors associated with vascular disease, smoking, obesity and diabetes are the risk factors for Alzheimer's disease. It is a slowly progressive disorder, with insidious onset and progressive impairment of episodic memory; instrumental signs include aphasia, apraxia, agnosia, together with general cognitive symptoms, such as impaired judgment, decision making and orientation. Behavioural signs, such as impaired judgement, decision-making and orientation. Behavioural signs, such as aggression, psychomotor agitation and psychosis (hallucinations and delusions), are very common in patients with Alzheimer's disease, especially in the late stages of the disease.

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PATHOPHYSIOLOGY

The neurological hall mall mark of AD includes deposits of amyloid fibrils in senile plaques and presence of abnormal tau protein filaments in the form of neurofibrillary tangles. Hippocampus, limbic system and cortex are the primary areas involved in the pathophysiology of AD.

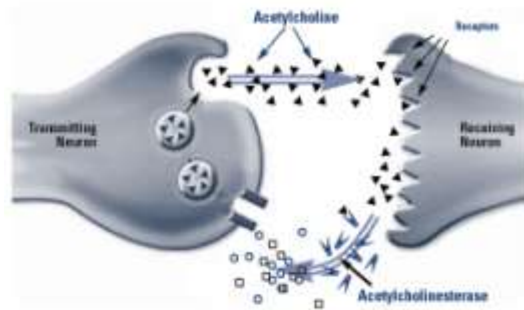

Fig:1 cholinergic hypothesis

1.2.1 Cholinergic deficits

According to the cholinergic hypothesis, memory impairment in patients suffering from AD is a result of decreased levels of the neurotransmitter, acetylcholine (Ach) in the cortex. The key role in the termination of nerve impulse transmission at cholinergic synapse by rapid hydrolysis of ach.

1.2.2 Senile plaque deposition

A major component of senile plaque is the amyloid ß (AD). Peptide which is formed as a result of proteolytic cleavage of the amyloid precursor protein (APP) by secretase. A ß peptide deposits or even the partially aggregated soluble form is responsible for triggering a neurotoxic cascade of events which ultimately result in neurodegeneration.

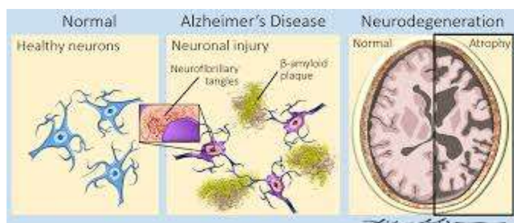

Fig:2 senile plaque depositions

1.2.3 Neurofibrillary tangle formation

Formation of intracellular neurofibrillary tangles which consist of hyper phosphorylated tau protein. Tau is an axonal protein which binds to microtubules and promotes their assembly and stability. Phosphorylation of tau protein is regulated by the balance between multiple kinases and phosphates. Hyperphosphorylation of tau protein and other microtubules are associated proteins,thus preventing the microtubules assembly and the impairing axonal transport systems leads to the neuronal death.

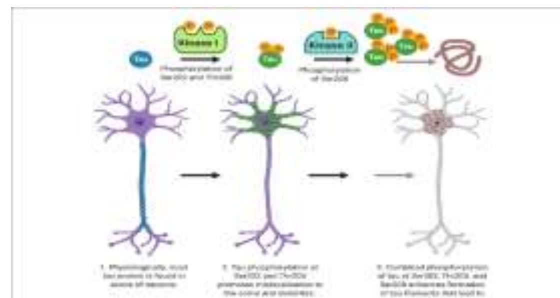

Fig: 3 neurofibrillary tangleformations

1.2.4 Oxidative stress

Oxidative stress in AD is the disturbance in metal homeostasis such as iron, copper, zinc, and aluminium capable of catalysing reactions that produce free radicals. Mitochondrial dysfunction is the source of ROS (reactive O**2** species) generation has been associated with a variety of degeneration pathway leading to AD progression.

1.2.5 Inflammatory cascade

Microglial activation leads to a massive production of inflammatory cytokines, ROS, and reactive nitrogen species, thereby contributing oxidative damage. Aß - peptides are another source for oxidative damage producing neurotoxic neurotoxic effects. Directly by inducing more ROS and indirectly by activating microglial leads to inflammation. Inflammation progresses the AD.

Fig:4 Alzheimer's disease pathology

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1.3.2 DRAWBACKS OF CURRENTLY USED DRUGS:

1. All the drugs that are currently used for Alzheimer's disease in the market are used to help manage the symptoms but not cure the disease.

2. Drugs act by only one mechanism therefore more than one drug is needed to be prescribedfor the patients. 3. Along with the desired therapeutic action all the drugs have many side effects.

1.4 PLANTS OF POTENTIAL INTERST IN ALZHEIMERS DISEASE THERAPY

Ever since the birth of mankind there has been a relationship between life, disease and plants. Primitive men started studying diseases and treatments. The most common thing they could find was there in environment i.e., the plants and animals. They started using plants and found that majority of plants were suitable as food, where as other were either poisonous or medicinally useful. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last decade has seen a major increase in their use in the developed world. In the Ayurvedic system, of medicine "Medhya drugs" -a group of herbal medicines are known for their actions on the nervous system. These "Medhya drugs" mentioned in Ayurvedic texts are said to improve mental abilities.

DRUG NAME	SIDE EFFECTS
Namenda® (Memantine)	Dizziness, headache, constipation, confusion
Razadyne® (Galantamine)	Nausea, vomiting, diarrhoea, weight loss, loss of appetite.
Aricept® (Donepezil)	Nausea, vomiting, diarrhoea.
Exelon® (Rivastigmine)	Nausea, vomiting, diarrhoea, weight loss, loss of appetite, muscle weakness

1.3 Treatments for Alzheimer's disease:
1.3.1 Available drugs in themarket:

DRUGNAME	DRUG TYPE	USE	MECHANISM OF ACTION
Namenda® (Memantine)	N-Methyl D-Aspartate Antagonist	Prescribed to treat symptoms of moderate to severe Alzheimer's Disease.	Blocks the toxic effects associated with glutamate and regulates glutamate activation.
Razadyne® (Galantamine)	Cholinesterase Inhibitor	Prescribed to treat symptoms of mild to moderate Alzheimer's Disease.	Prevents the breakdown of acetylcholine by inhibiting Acetylcholinesterase enzyme and stimulates nicotinic receptors to release more acetylcholine.
Aricept® (Donepezil)	Cholinesterase inhibitor	Prescribed to treat symptoms of mild to moderate and moderate to severe Alzheimer's Disease.	Prevents the breakdown of acetylcholine.
Exelon® (Rivastigmine)	Cholinesterase Inhibitor	Prescribed to treat symptoms of mild to moderate Alzheimer's Disease.	Prevents the breakdown of acetylcholine and butyrlcholine in the brain.

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1.4.1 MEDICINAL PLANTS
1. Acorus calamus (Araceae),
2. Allium sativum (Alliaceae)
3. Angelica sinensis (umbeliferae)
4. Astragalus membranaceus (Fabaceae)
5. Bacopa monnieri (Scrophulariaceae)
6. Boerhaaviadiffusa (Nyctaginaceae)
7. Baliospermummontanum (Euphorbiaceae)
8. Convolvulus pluricaulis (Convolvulaceae)
9. Centella asiatica (Apiaceae),
10. Celastruspaniculatus (Celastraceae)
11. Coelogyne evalis (Orchidaceae)
12. Clitoriaternatea (Fabaceae)
13. Curcuma longa (Zingiberaceae)
14. Desmodiumgangeticum (Fabaceae)
15. Dipsacus asper wall (Dipsacaceae)
16. Glycyrrhiza glabra (Fabaceae)
17. Hypericum perforatum (Hypericaceace)
18. Indigo naturalis (Apiaceae)
19. Lycoris radiata (Amaryllidaceae)
20. Moringa oleifera (Vitaceae)
21. Nicotiana tadaccum (Solanaceae)
22. Piper longum (Piperaceae),
23. Polygala tenuifolia (Polygalaceae)
24. Radix polygonimultiflori (Polygalaceae)
25. Sidaspinosa (Malvaceae)
26.Semecarpusanacardium (Anacardiaceae)
27.Tinospora cordifolia (Menispermaceae)
28. Vigna radiate (Fabaceae)
29. Vitis vinifera (Vitaceae).
30. Withaniasomnifera (Solanaceae).

II. DRUG PROFILE

2.1BACOPA MONNIERI

Bacopa monnieri is also known as Bramhi ghratam, it is an herb from India. It is also called as Indian pennywort, Water hyssop, herb of grace it belonging to the Scrophulariaceae family.it is called as Aindri& Bramhi in Sanskrit. It is another type of apoptogenic herb that also has #nootropic effects and anti- anxiety properties. It's a complementary and alternative medicines (CAM) have been widely used throughout history. After 3000 years of practice, the Ayurveda medicinal system is one of the oldest health care systems in the world, promoting a holistic view of health and prescribing individualized treatments. One common CAM treatment deriving from the Ayurveda medicinal system is B. monnieri (James D. Kean et al.,2016). In Ayurvedic medicine as it strengthens memory and intellect(Medhya;2006). Board of National Medicinal Plants reported that estimated annual market demand of B. monnieri was 1000 -2000 tons per year due to memory revitalizing/enhancing capacity. It has continued to increase day by day to treat the numerous disorders & it has wide spectrum of pharmacological activities against Alzheimer's disease.

Fig: 5 Bacopa Monnieri

2.1.1BOTANICAL CLASSIFICATION
Common name: Brahmi
Botanical name: Bacopa monnieri
Biological source:Bacopa monnieri leaves
Family: Scrophulariaceae
Class: Magnoliopsida
Order: Scrophulariales
Division: Magnoliophyta
Kingdom: Plantae
Chemical constituents: Flavonoids, Phenoilthenoid, Saponins, Cucurbitacins.

2.1.2. PLANT DESCRIPTION

This herbaceous plant grows widespread in tropical, swampy areas. The entire B. Monnieri plant, including leaves, stems, flowers, is utilized in Ayurvedic preparations. B. Monnieri is a lowering that typically reaches a height of 10-30cm.The stems of B. Monnieri are slender succulent, and often reddish or greenish in colour. The leaves are small succulent, and oblong-shaped, with rounded tips & arranged oppositely along stems typically with 1-2cm length & leaves has a glossy texture & dark green in colour. The flowers are five petaled &tiny which are white or purple in colour rand arise from leaf axils. B. Monnieri develops small ovoid fruits containing seeds. The roots of B. Monnieri are fibrous &shallow spreading horizontally near the soil surface. They help anchor the plant & absorb the nutrients & water from surrounding soil.

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2.1.3 COMMON NAMES OF B. MONNIERI

Telugu: Saraswathi Aaku
Bengali: Brahmishak
Sanskrit: Trayanthi
Tamil: Nirpirami
Hindi: Jalbuti

2.1.4 ACTIVE CHEMICAL CONSTITUENTS

B.Monnieri contains several chemical constituents, of which the most important are Flavinoids, proanthocyanidin, phenolic compounds,Terpinoids, Herpestine, Saponins A, B, C, Stigmastanol, Partic acid, Glutamicacid,Pseudojujubogenin glycoside.

2.1.5 PHARMOCOLOGICAL USES

Mental disturbances
Brain & Nervous tonic
Promotes mental calm & clarity
Insomnia
Inflammations

2.1.6 TRADITIONAL USES

B. Monnieriis used in the memory improvement, insomnia, epilepsy, and as an anxiolytic memory acquisition,
anxiety reduction with using Bacopa.

2.2 MORINGA OLEIFERA

M.Oleifera is a plant native to northern india that can also grow in other tropical &subtropical places, like Asia and Africa. Folk medicine has used the leaves, flowers, seeds, and Roots, of this plant for centuries. It's Commonly known as Drumstick tree, Horseradish Tree, in Sanskrit it is called Shirgu, Shobhanjana, Sahijna, Munaga. It is native to the Indian subcontinent, but it has been widely cultivated throughout Africa for over 2000 years. It is particularly popular in the sahel region of west Africa where it is grown as a food crop, a source of fuel, and a natural medicine but it is alsogrown as a food crop, a source of fuei, and a natural medicine but it is also grown plenty all over east Africa. Moringa was discovered in Northern India around 2000 BC. Traditional doctors quickly discovered its medicinal impact and called it "The Miracle Tree.

2.2.1 BOTANICAL CLASSIFICATION

Common Name: Drumstick
Botanical Name:Moringa oleifera
Fig: 6 Moringa Oleifera
Biological Source:Moring oleifera leaves
Family:Vitaceae
Class:Magnoliopsida
Order:Vitalas
Division:Magnoliophyta
Kingdom: Plantae
Chemical Constituents:Flavonoids, Glycosides, Saponins, Terpinoids etc.

2.2.2 PLANT DESCRIPTION

This is a fast-growing, deciduous tree that can reach a height of 10-12m (33-39ft)and trunk diameter of 45cm. The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish -white,hairy bark. The Tree has an open crown of dropping fragile branches, and the leaves build up a feathery foliage of tripinnate dark green leaves. The fruit is a hanging, three-sided brown 20-45cm capsule, which holds green colour globular seeds with a diameter around 1cm. The seeds have three whitish papery wings and dispersed by wind and water. The flowers are fragrant and hermaphroditic, surrounded by five unequal, thinly veined,Yellowish -white petals. They grow on slender, hairy stalks in spreading or drooping flower clusters.

2.2.3 COMMON NAMES OF M. OLEIFERA

Telugu: munaga
Sanskrit: Subhanjana
Tamil: Mulaga
Hindi: Saguna

2.2.4 ACTIVE CHEMICAL CONSTITUENTS

M. Oleifera contain several chemical constituents, of which the most important are Flavonoids, Saponins, Glycosides, Terpinoids, Alkaloids, Antioxidants, Antimicrobial & Anti-carcinogenic Anti-inflammatory.

2.2.5 PHARMOCOLOGICAL USES

Restore mono amine levels of brain
CNS Activity
Neuro protective
Spatial memory
Anti-inflammatory

Anti-oxidant

2.2.6 TRADITIONAL USES
In South Asia and Africa as a traditional folk medicine to treat many ailments such as paralysis, helminthiasis, sores and skin infections.

2.3VITIS VINIFERA
Vitis vinifera is the common grape wine is a species of flowering plant, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Por North-southern Germany & East to Northern Iran. There are currently b/w 5000 & 10000 varieties of Vitis vinifera grapes though only a few are of commercial significance for wine.

Fig 7 Vitis vinifera

2.3.1 BOTANICAL CLASSIFICATION
Common Name: Grapes
Botanical Name:Vitis vinifera
Biological Source:Vitis vinifera seeds
Family:Vitaceae
Class:Magnoliopsida
Order:Vitalas
Division:Magnoliophyta
Kingdom: Plantae
Chemical Constituents:Proanthocyanidin, Phenolic compounds, Flavonoids etc.

2.3.2 PLANT DESCRIPTION
It is a liana growing 12-15cm (39-49ft) tall at a fast rate. Having a flaky bark, its leaves are alternate,polymetricallylobed, deciduous, dark green on top & light green on below attached to supports tendrils.Stems called twigs, grow through their tip, cauline apex. Roots usually sink to depth of 2-5mts sometimes 12-15 mts. Their flowers small greenish to white are grouped inflorescences. Their fruits are different in shapes depending on subspecies, berries grouped into clusters they may present in dark blueor greenish usually 2 locular with 5 seeds in wild specices it is 6mm(0.24in) in diameter. In cultivated plants usually much larger up to 3cm (1.2in) long &green, redor purple.

2.3.3 COMMON NAMES OF VITIS VINIFERA
Telugu: Draksha
Bengali: Angura
Sanskrit: Mridvika
Hindi: Angoor

2.3.4 ACTIVE CHEMICAL CONSTITUENT
Vitis vinifera contain several chemical constituents of which the most important are
Proanthocyanidin, Phenolic compounds, Flavonoids, Saponins, Terpinoids, vitamins, Anthocyanins, organic acids.

2.3.5 PHARMOCOLOGICAL USES
Counteract the neuro degenerative damage of Alzheimer's disease
Short term memory treated
Cognitive behaviour is treated
Anti-oxidant
Anti-inflammatory

2.3.6 TRADITIONAL USES
Vitis vinifera are traditionally used to treat Alzheimer disease, Diabetes (improving blood sugar control)Improving night vision, Protecting collagen and elastin in skin (anti-aging),
Treating haemorrhoids, Protecting against oxidative rancidity and bacterial pathogens.

2.4 ZEBRA FISH[DANIO RERIO]
2.4.1 SCIENTIFIC CLASSIFICATION:
Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Division: Cypriniformes
Family: Cyprinidae
Genus: Danio
Species: D.rerio

Fig.9 Male and Female zebra Fishes

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Viable animal models (i.e. rodent and primate) have enabled researchers to infer about the fundamental features of human behaviour and physiology. Since the zebra fish's introduction a model for neural development by Streisinger in the 1960's (Grunwald & Eisen, 2002), its preenise as a genetic tool for biological research has nearly been realized (Beis&Stainier, 2006; Driever et al., 1996; Guo, 2004; Haffter et al., 1996). Recent years, however, have seen a steady increase in the use of this species in behavioral applications (Miklósi& Andrew, 2006, Spence, Gerlach, Lawrence, & Smith, 2008). Many features of the zebrafish make it a particularly attractive candidate for inferring higher-level vertebrate behaviour the zebrafish (Danio rerio) has been at the forefront of neurobiological research and is steadily gaining favour as a model for behavioral applications. The ease of handling, high yield of progeny, and efficient mode of drug delivery make this species a particularly useful model for behaviour.

Recently, **Zebrafish (Danio rerio)**grabbed the attention as a model of aging. Many studies can be performed include teratogenicity studies, Cardiovascular system studies, Hyperlipidemic studies, mutagenicity studies. Zebra fish do not undergo metamorphosis, the continuous process of development, maturation and aging without any interruption favours the study of age related processes. Studying the impact of environmental factors and pharmacological agents in zebrafish is relatively easy. These animals have well-developed sensory organs, detect diverse environmental stimuli, and show well-defined behavioural responses to them. Zebrafish skin or gills provide a gateway for many soluble agents. As a result, the majority of biologically active compounds can be administered non-invasively, simultaneously and in precise concentrations directly into the water surrounding hundreds of embryos, larvae or adult zebrafish.

2.4.2 USE OF FISH MODELS IN BEHAVIORAL NEUROSCIENCE:

Fish are the obvious ancestral form of existing tetrapods, so it is not terribly surprising to find that they show most of the behaviour seen in terrestrial species in some form or other. In social behaviour alone, there are species known to show monogamous mating for life (e.g., angelfish,individual recognition of conspecifics by sight or odor), socially mediated learning, intricate mate- selection strategies.

With respect to cognition and adaptive behaviour, fish show highly developed spacial navigation abilities, non-associative learning such ashabituation, precise timing abilities, Pavlovian conditioning, operant behaviour motivated by aversive stimuli such as shuttle box behaviour negatively reinforced avoidance, and food-reinforced lever pressing positively reinforced responding.

In terms of sensory processes, fish have excellent color vision some species generate and detect weak electrical currents, a sense that they use to detect predators and prey (Colwill RM et al., 2005) and have lateral-line organs that allow them to resolve the location, size, and features of distal objects by sensing their pressure shadows.

Behavioural research with fish began with ethologists and comparative psychologists asking questions about the evolution of learning, cognitionand brain function. As in other species, the understanding of the teleost bram has been driven in large part by the development of appropriate behavioral assays. The extent to which basic behavioral and brain processes in mammals and fish are analogous remains an open question there are clear similarities and differences and, as with all animal models, the validity of a fish model hinges on the particular question being asked.

Zebrafish have rapidly become a prominent model for studying the molecular basis of vertebrate neurodevelopment. The clear chorion of the zebrafish allows continuous visualization of neuroanatomy, their rapid development and accessibility to genetic analysis make the zebrafish an excellent model system for molecular and mechanistic studies of neurodevelopment. Since its introduction, many genetic mutants have become available, including varieties that can help determine the molecular mechanisms of neurobehavioral function. More recently, the availability of morpholino techniques, whereby specific parts of the genome can be reversibly suppressed during early development, provides a unique way to explore the molecular biology of development. Zebrafish have been critical in the identification of a variety of genes affecting Farious aspects of neural development and function. As a result, the genetics and physiology of learning and memory are now being more widely studied.

Zebra fish Many tasks are now able to top behavioural processes only only studied with rodents and goldfish.

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2.4.3 ADVANTAGES OF ZEBRA FISH AS A MODEL FOR PRECLINICAL STUDY

- The vertebrate zebra fish genome and anatomy show only - 420 million years (Myr) of divergence from the human lineage rather than the-600 million years Myr of the ecdysozoans (Drosophila and C. elegans. Therefore, most human genes have clearly identifiable orthologues in zebra fish.
- The numerous and externally fertilized embryos of zebra fish are easily amenable to methods for manipulating gene and protein activity such as injection of antisense oligonucleotides, mRNAs or transgenes.
- Zebra fish possess genes orthologous to all the genes known to be involved in Alzheimer's disease. The genes appa and appbare duplicates of an ancestral teleost orthologue of human APP. The genes psent and psen2 are orthologues of human PSENI and PSEN2, respectively.

III. AIM

The main aim of this study is to enhance the learning memory and neuroprotection of scopolamine induced Alzheimer’s in zebra fish with poly herbal plant extract.

3.1 OBJECTIVES

1. To collect the leaves of Bacopa monnieri and Moringa oleifera and shade dry them.
2. To collect the seeds of Vitis vinifera and dry in shade.
3. To carry out extraction for theleaves of Bacopa monnieriand Moringa oleifera using Maceration extraction using ethanol as a solvent.
4. To carry out the extraction process of Vitis vinifera seeds by maceration extraction process.
5. To acclimatize the zebra fish to the laboratory conditions.
6. To train the zebra fishes for the testing of the prepared poly herbal drugs and the selected standard drugs.
7. To induce the symptoms of Alzheimer’s disease in zebra fish by administering scopolamine by placing the fish in the scopolamine solution.
8. To treat the zebra fishes by administering the poly herbal plant extract orally.
9. To determine the toxicity of the poly herbal plant extract in zebra fish.
10. To carry out the behavioural assessments in zebra fish.

- Inhibitory avoidance Test
- T-Maze Test

IV. EXPERIMENTAL WORK

4.1 MATERIALS AND METHODS

4.1.1 ANIMALS

Adult Male Zebra Fishes were purchased from a local aquarium pet store. Animals were kept in housing tanks with the Normal fresh water of 5 animals per 3 litres. Animals are kept at 14-10hr light and dark photoperiod and are continuously aerated Animals were fed with pellets obtained from the pet store. Animals were acclimatized to the laboratory conditions for about 10 days before the starting of the experiment. All experiments were carried out according to the guidelines of the Institutional Animal ethics committee [IAEC]. Animal ethics no. IAEC/KVSP/O2/7/2024.

4.2 Collection And Authentification Of Herbal Plants

Plants of Bacopa monneirii, Moringa oliefera, and Vitis vinifera was collected from the villages Nannur and Devamada, Kurnool. Authentification was performed by K. Vanitha kumari head of the dept. Botany st.Joseph’S degree college, Kurnool. The specimens voucher no. are 0111, 0622, 0625 they are identified as Bacopa monnieri, belonging to the family Scholphulariaceae, Moringa olieferaand Vitis vinifera belonging to the familyVitiaceae.

4.2.1 EXTRACTION OF HERBAL PLANTS

4.2.2 METHODS

4.2.2.1 Bacopa monnieri

50gms of leaf powder of Bacopa monnieri was extracted with ethanol using Maceration extraction process. The powder is added with ethanol until the leaf powder gets submerged within the solution and left undisturbed for 3-4 days. Then the extract was evaporated at 60-65 degrees, dried plant extract. The extract was collected and stored in the airtight container.

4.2.2.2 Moringa oliefera

50gms of leaf powder of Moringa olieferawas extracted with ethanol using Maceration extraction process. The powder is added with ethanol until the leaf powder gets submerged within the solution and left undisturbed for 3-4 days. Then the extract was evaporated at

60-65 degrees, dried plant extract was collected and stored in the airtight container.

4.2.2.3 Vitis vinifera

50gms powder of the seeds of Vitis vinifera was extracted with ethanol using Maceration extraction process. The powder is added with ethanol until the leaf powder gets submerged within the solution and left undisturbed for 3-4 days. Then the extract was evaporated at 60-65 degrees, and dried plant extract was collected and stored in the airtight container.

4.3 PHYTOCHEMICAL SCREENING

Phytochemical analysis was carried out for extracted plant materials. Individual plant extract was taken and tested for presence of alkaloids, tannins, glycosides, phenols, Flavonoids, carbohydrates, terpines, diterpines, steroids, and aminoacids and proteins.

4.3.1 Test for Alkaloids

The extract were treated with diluted HCL and filtered. The filtrate was treated with various alkaloidal agents.

- **Mayer's Test:** Sample was treated with Mayer's reagent; appearance of cream colour indicates the presence of Alkaloids.
- **Dragendroff's Test:** Sample was treated with Dragendroff's reagent, appearance of reddish brown precipitate indicates the presence of Alkaloids.
- **Hager's Test:** Sample was treated with Hager's reagent; appearance of yellow colour indicates the presence of Alkaloids.
- **Wager's Test:** Sample was treated with wager's reagent; appearance of brown colour precipitate indicates the presence of Alkaloids.

4.3.2 Test for Carbohydrates

The extracts was treated with 3 ml of alpha naphthol in alcohol and conc. Sulphuric acid was carefully added to the side of the test tubes. Formation of a violet colour ring at the junction of two liquids indicates the presence of carbohydrates.

- **Fehling's Test:** To the sample Fehling's solution A and B was added and heated for two minutes. Appearance of reddish brown precipitate indicates the presence of reducing sugars.
- **Benedict's Test:** To the sample Benedict's solution was added and heated for two minutes. Appearance of reddish orange precipitate indicates the presence of reducing sugars.
- **Barford's Test:** To the sample Benedict's solution was added and heated for two minutes. Appearance of reddish orange precipitate indicates the presence of reducing sugars.

4.3.3 Test for Proteins:

- **Biuret's Test:** To the extracts copper sulphate solution followed by sodium hydroxide solution, a violet colour precipitates indicates the presence of Proteins.
- **Million's Test:** To the extracts million's reagent was added, appearance of pink colour indicates the presence of Proteins.

4.3.4 Test for Steroids:

- **Libermann Burchard's Test:** The extract weretrated with conc. Sulphuric acid and glacial acetic acid followed by acetic anhydride, a violet colour ring appears at the junction of the liquids and appearance of green colour in the aqueous layer indicates the presence of steroids.

4.3.5 Test for Sterols:

- The extracts were treated with 5% KOH solution; appearance of pink colour indicates the presence of sterols.

4.3.6 Test for Phenols:

- The extracts were treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenols.
- The extracts were treated with 10% sodium chloride solution, appearance of cream colour indicates presence of Phenols.

4.3.7 Test for Tannins:

- The extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates presence of Tannins.
- The extracts were treated with aqueous bromine water, appearance of white precipitate indicates presence of Tannins.

4.3.8 Test for Flavonoids:

5ml of the extracts solution was hydrolysed with 10% Sulphuric acid and cooled. It was then extracted with diethyl ether and divided into 3 portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1n sodium hydroxide and 1ml of diluted ammonia solutions were added to the first second and third test tube

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respectively. Development of yellow colour in each test tube indicates the presence of flavonoids.

Shinoda's Test: The extracts were dissolved in alcohol, to whi9ch a piece of magnesium followed by drop wise addition of conc. HCL and heated. Appearance of magenta colour indicates the presence of flavonoids.

4.3.9 Test for Gums and Mucilage:

The extracts were treated with 25 ml of absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

4.3.10 Test for Glycosides:

A pinch of the extract were dissolved in glacial acetic acid and few drops of ferric chloride solution was added followed by the addition of conc, sulphuric acid formation of red ring at the junction of the two liquids indicates the presence of glycosides.

4.3.11 Test for Saponins:

Foam Test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam at the upper part of the test tubes presence of saponins.

4.3.12 Test forTerpines:

The extracts were treated with tin and thionyl chloride, appearance of pink colour indicates the presence of terpines. (kokate et al 27th ed 2004).

4.4 PREPARATION OF POLY HERBAL PLANT EXTRACT

The three plant extracts was collected and mixed with ratio 1:1:1. The plant extract is individually weighed for dose of 2000mg/kg.

4.5 EVALUATION OF TOXICOLOGICAL STUDIES

4.5.1 Oral acute toxicity studies

Acute toxicity study is generally carried out for the determination of LD_{50} value in experimental animals. The LD_{50} determination was done in zebra fish by OECD guidelines 203. The aim of performing acute toxicity study is for establishing the therapeutic index of a particular drug and to ensure the safety in vivo. (Shanti Bhushan Mishra, et al).

4.6 INDUCTION OF ALZHEIMER'S DISEASE WITH SCOPOLAMINE

Scopolamine solution of 200µM was prepared. To prepare 200µM solution of scopolamine, 88mg of scopolamine was dissolved in 1000mg of water. Zebra fish was directly placed in the above scopolamine solution for 1 hour before commencing the experiment. (S.K. Richetti, et al., 2011).

4.7 RIVASTIGMINE

The dose of Rivastigmine for zebrafish was calculated based on the dose of Rivastigmine for rats in the previous study. According to the previous study, Rivastigmine dose for rats was 1.5mg/kg and in this study the same dose used but adjustedto the body weight of zebra fish. The average body weight of zebra fish is 0.5gm. So, dose of Rivastigmine calculated for 0.5 gm was 0.75 µg.

The maximum volume of solution that can be injected into the zebra fish 5µl. So, Rivastigmine solution was prepared so that 5µL contains 0.75µg.

To prepare Rivastigmine solution containing 0.75µg in 5µL, 1.5mg of Rivastigmine was dissolved in 1mL of water. 2.5µL is pipetted out into Rivastigmine in 25µL water was added to it. This solution contains 3.75µg Rivastigmine in 2.5µL water. From the above solution, 5µL is taken in a pipette and directly administered to zebrafish orally, where, 5µL contains 0.75ML of Rivastigmine.

4.8 EQUIPMENTS / MATERIALS USED:

4.8.1 Aquarium: A Glass aquarium of dimensions 30cmX15cmX15cm was used for housing zebra fishes at a density of 5 fishes per 3 litres.

4.8.2 Aerator: Aerators of Sobo aquarium air pump-SB-548A were used for supplying air into the water in order to make the fishes stay alive.

4.8.3 Animal Feed: Zebra fishes were fed with dry food which was regularly provided in the aquarium pet shop. The dry food contains crude protein (30%), crude fat (4%) crude fibre (5%), crude ash (12%), moisture (10%).

4.8.4 Micro Pipette: Micropipettes of company Thermo scientific fine pipette were used and of capacity 10µL and 100µL were used during the study.

4.8.5 Inhibitory avoidance Test Apparatus:

An aquarium of dimensions 18cmX9cmX7cm was prepared using acrylic glass sheet. A sliding partition was made in middle of the aquarium.

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Fig.9 PASSIVE AVOIDANCE TEST

4.8.6 T Maze Test Apparatus:
A T-Shaped maze was prepared using acrylic glass sheet of dimensions 50cmX10cmX10cm long arm and 2 short arms of dimensions 20cmX10cmX10cm. A start box of 10cmX10cmX10cm was prepared in the long arm.

Fig.10 T MAZE TEST APPARATUS

4.9 ANIMAL PROCEDURES:
Dose of the poly herbal plant extract was chosen based on the acute oral toxicity studies done on zebrafish. According to acute oral toxicity studies conducted on zebrafish poly herbal plant extract lethal dose is found to be 2000 mg/kg. From the lethal dose effective dose is determines as (200mg/kg,133mg/kg, and 80mg/kg and 40mg/kg). As per the weight of zebrafish test drug was administered orally using 1-10µl Micro-Pipette. Standard drug used in the present study was Rivastigmine, which is an Acetylcholinesterase inhibitor, currently used in the market for treating patients with Alzheimer's disease. Water is used as a vehicle: both standard drug and poly herbal plant extract were dissolved/suspended in the water. Before 2 hours of the commencement of the experiment, standard Rivastigmine and test drug were administered to the animals and 1 hour before the commencement of the experiment zebrafishes were treated with scopolamine was prepared and animals were transferred to the tank containing the scopolamine solution before 1 hour of beginning of the experiment. Standard and test drug solutions were prepared freshly on the day of the experiment.

4.10 BEHAVIORAL ANALYSIS:
In the present study, to evaluate the disease symptoms 2 behavioural tests were performed. Inhibitory Avoidance Test and T-maze test.

4.11.1 APPARATUS:
An aquarium of dimensions 18cmX9cmX7cm was prepared using acrylic glass especially for performing this evaluation test. This tank was divided in to 2 equal white and dark compartment by a sliding door (9cmX7cm). Dark compartment was made by covering the three sides of the tank with the black sheet. Tank was filled with water level up to 3cm and sliding partition was raised 1cm above the floor to allow the fishes move freshly from one compartment to other. In the dark compartment, two electrodes were kept extending the tank height in the two concerns of tank and 3mA current was applied (Martina Blank et al., 2009).

4.11.2 EXPERIMENTAL PROCEDURE:
Inhibitory Avoidance Test consists of training session and test session. In the training session, animals were allowed to learn a condition and in the test session, animals were evaluated for their memory.

4.11.3 TRAINING SESSION:
In the training session, animals were kept in the white compartment while the portion is closed. After 1 minute, the partition was raised 1cm above the tank floor and fish was allowed to enter the dark compartment. After the entry of fish in to dark compartment (with its whole body inside the compartment) sliding door was closed and then a current shock of 3mA was applied for 2 to 3 seconds. Fish was then removed from the apparatus and transferred to the housing tank. This procedure was followed to train all the fishes. Before two hours of beginning of the training session, standard and test drug were administered and 1 hour before the training, fishes were treated with 200µM scopolamine solution. (Martina Blank et, al.,2009).

4.11.4 TEST SESSION:
After 24 hours of the training session, animals were tested.

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In the test session, the same procedure as in training session was followed expect the shock of 3mA was not applied. Fishes were remove immediately after their entry into the dark compartment.

4.11.5 PARAMETER TO BE EVALUATED:
Latency to entry the dark compartment was measured in both training and test sessions.

4.12 T MAZE TEST
4.12.1 APPARATUS:
T maze was prepared using transport acrylic glass. The dimensions of the maze are 50cmX10cmX10cm of stem of the maze and the two arms are of dimensions 20cmX10cmX10cm. At the foot of the stem 10cmX10cmX10cm start box was prepared using a sliding door. Water in the maze was filled up to 6cm height and temperature of water was maintained at 28°C throughout the experiment. Blue and green coloured sleeved were fitted around three sides of the arms. In green coloured arm, fish was awarded with the food only during training session.

4.12.2 EXPERIMENTAL PROCEDURE

4.12.3 TRAINING SESSION:
Initially, animals are placed in the T maze without any coloured sleeves for habituation to the T maze before commencing the session. Training session should be carried for 6 consecutive days. In the training session, animal is placed in the start box for 1 min. After 1 minute, sliding door was opened to allow the fish to move from start box. Following its exit, start box is closed. Once fish entered in any of the arms another door was used to prevent the fish enter into the stem of the maze. Fish was then observed for 4 minutes.

4.12.4 TEST SESSION:
On the 7th day i.e., after 6 days of training session, test session is carried out. In the test session, the same procedure was followed as in training session except the awarding of food in the green coloured arm.

4.12.5 PARAMETERS TO BE EVALUATED:
Both training session and test sessions, the following parameters are evaluated.
1. Total No. of entries.
2. Time spent in green arm.

4.1.2 CHEMICALS:

CATEGORY	CHEMICALS USED
Vehicle	Water
Disease inducing agent	Scopolamine
Standard Drug	Rivastigmine
Test drug	Poly herbal plant extract (PHPE)

Table no. 4 materials used

4.1.3 EXPERIMENTAL DESIGN

S.NO	GROUP	NO. OF ANIMALS	TREATMENT
1	I	5	Normal control
2	II	5	Control-scopolamine(200µm dissolved in water for 1 hour).
3	III	5	Scopolamine+Rivastigmine
4	IV	5	Scopolamine+PHPE(200mg/kg,p.o)
5	V	5	Scopolamine+PHPE(133mg/kg,p.o)
6	VI	5	Scopolamine+ PHPE (80mg/kg,p.o)

Table no. 3 experimental design

V. RESULTS AND DISCUSSIONS

5.1 PRELIMINARY PHYTOCHEMICAL SCREENING

The individual plant extract is taken and assayed for presences of chemical constituents. The results about phytochemical screening are shown in the following table.

S.NO	PHYTO-CHEMICAL TEST	PLANTS		
		Bacopa monnieri	Moringa oleifera	Vitis vinifera
1	Test for Alkaloids	+ve	+ve	-ve
	Mayer's test			
	Dragendroff's test			
	Wagner's test			
2	Test for Tannins	+ve	+ve	+ve
3	Test for cardiac glycosides	-ve	-ve	-ve
4	Test for Sterols	+ve	+ve	+ve
5	Test for Phenols		+ve	+ve
6	Test for Flavonoids	+ve	+ve	+ve
7	Test for Carbohydrates	+ve	+ve	+ve
	Molish test			
8	Test for Glycosides	+ve	+ve	+ve
9	Test for Diterpines	+ve	+ve	+ve
10	Test for Aminoacids and Proteins	+ve	+ve	+ve
11	Test for Terpines	+ve	+ve	-ve
12	Test for Saponins	+ve	+ve	-ve
	Solkowski test			

Table no.5 phytochemical screening

5.2 ORAL TOXICITY STUDIES

Oral acute studies were performed to determine the therapeutic index of a drug and to ensure the safety of in in vivo. The LD_{50} of the poly herbal plant extract were found to be 2000mg/kg. Results were shown in the following table.

S.NO	NO. OF ANIMALS	DOSES	NO. OF ANIMALS DIED
1	5	5mg/kg	0
2	5	50mg/kg	0
3	5	300mg/kg	0
4	5	3000mg/kg	0

Table no. 6 Oral acute toxicity studies

5.3 INHIBITORY AVOIDANCE TEST

Zebra fish were first evaluated for learning ang memory using Inhibitory avoidance test. In this test, latency period (in seconds) to cross the aperture of 1cm into dark compartment was noted. Latency time of training and test session were compared to find differences between them. All statistical analysis were done using graph pad prism software and data were analysed using one-way ANOVA and data were presented as mean±SEM. Latencies of multiple groups were compared using Dunnet's multiple comparison test.

Scopolamine was used for memory impairment and its effectiveness was demonstrated bv treating a group of zebra fishes with scopolamine (200Mm dissolved in water for 1 hour). The comparison of latency of normal and scopolamine (graph 1) showed that there is a great retention of memory in zebra fishes of normal group whereas there is scopolamine treated group showed a hindrance in the memory retention. The latency period of training sessions in the both groups was found to be the same.

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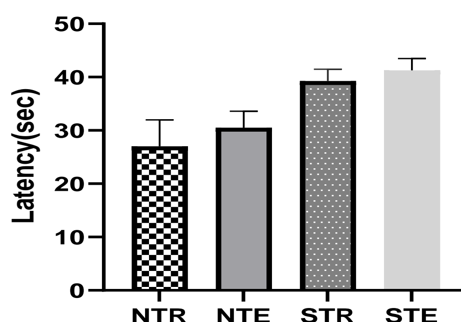
S.NO	GROUPS	LATENCY		MEAN	MEAN±SEM
		TRAIL I	TRAIL II		
1	NTR	30	30	27	27±1.62
		21	21		
		25	25		
		32	32		
2	NTE	32	32	30.5	30.5±1.017
		29	29		
		27	27		
		34	34		
3	STR	40	40	39.25	39.25±0.72
		37	37		
		42	42		
		38	38		
4	STE	42	42	36.5	36.5±1.80
		40	40		
		34	34		
		30	30		

Table 7: The latency period of Normal (water only) and only scopolamine treated groups were tested. The average latency periods (rounded off to nearest whole number) and SEM was calculated.



Graph: 1 Graph showing the Latency period (in sec) of Normal (Water only) and Scopolamine (Positive control) groups in Training and Test sessions.

The effectiveness of the poly herbal plant extracts was evaluated by combining with the scopolamine exposure before training and test sessions. The training and test latencies of normal and scopolamine groups of poly herbal plant extracts were compared with the training and test latencies of normal and scopolamine groups of standard (RIVASTIGMINE) group (graph 2). Initially, poly herbal plant extracts was administered before 1 hour of scopolamine treatment. Then zebrafishes were exposed to scopolamine before 1 hour of training and test sessions. The comparison of latency of standard and test groups showed that the training and test normal groups showed higher retention in memory. Normal Rivastigmine test group. The PHPE II (PTE II) and PHPE III (PTE III) was showed lesser retention in memory compared to normal PHPE I

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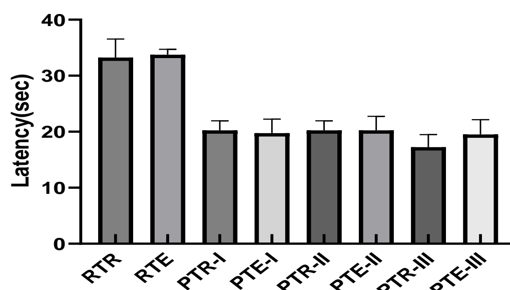
group, The Rivastigmine and PHPE I (PTE I) test latencies were higher than the training latencies i.e., Rivastigmine and PHPE I (PTE I) test groups showed higher retention in memory than the training latencies (graph 3).



Graph 2: Graph showing the Latency period of normal Rivastigmine groups and Normal PHPE groups in Training and Test sessions.

S.NO	GROUPS	LATENCY		MEAN	MEAN±SEM
		TRAIL I	TRAIL II		
1	RTR	13	13	25.25	25.25±4.81
		17	17		
		46	46		
		25	25		
2	RTE	30	30	50	50±7.07
		50	50		
		80	80		
		40	40		
3	PTR-I (200mg/kg)	28	28	31.75	31.75±4.02
		49	49		
		30	30		
		20	20		
4	PTE-I (200mg/kg)	48	48	59.25	59.25±7.02
		89	89		
		60	60		
		40	40		
5	PTR-II (133mg/kg)	12	12	22.75	22.75±2.63
		30	30		
		22	22		
		27	27		
6	PTE-II (133mg/kg)	12	12	19.75	19.75±2.45
		28	28		
		24	24		
		15	15		
7	PTR-III (80mg/kg)	18	18	24	24±1.92
		20	20		
		30	30		
		28	28		
8	PTE-III (80mg/kg)	17	17	20.5	20.5±2.59
		32	32		
		19	19		
		14	14		

Table 8: The Latency periods of normal Rivastigmine and PHPE groups. The average latency periods (rounded off to nearest whole number) and SEM was calculated.

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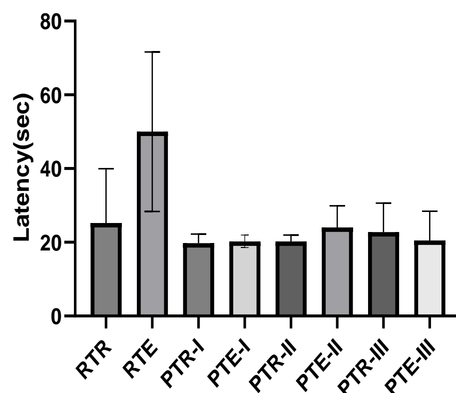

Graph:3 Latency periods of scopolamine treated Rivastigmine group and scopolamine treated PHPE groups.

S.NO	GROUPS	LATENCY		MEAN	MEAN±SEM
		TRAIL I	TRAIL II		
1	SRTR	19	19	27.25	27.25±4.62
		12	12		
		42	42		
		36	36		
2	SRTE	44	44	44	44±1.92
		36	36		
		46	46		
		50	50		
3	SPTR-I (200mg/kg)	30	30	24.5	24.5±1.75
		27	27		
		19	19		
		22	22		
4	SPTE-I (200mg/kg)	12	12	25.25	25.25±3.26
		28	28		
		25	25		
		36	36		
5	SPTR-II (133mg/kg)	27	27	26.5	26.5±2.98
		39	39		
		22	22		
		18	18		
6	SPTE-II (133mg/kg)	35	35	28.25	28.25±3.80
		40	40		
		24	24		
		14	14		
7	SPTR-III (80m/kg)	18	18	25	25±2.18
		23	23		
		34	34		
		25	25		
8	SPTE-III (80mg/kg)	38	38	31.25	31.25±3.56
		43	43		
		43	43		
		21	21		

Table 9: The Latency periods of scopolamine treated Rivastigmine group and scopolamine treated PHPE groups.

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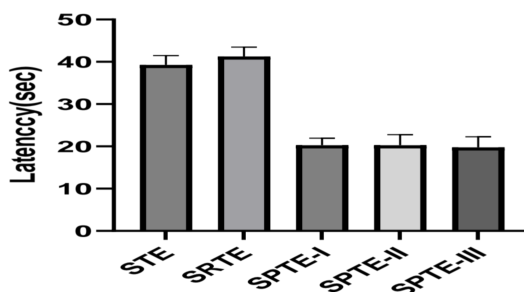
Test latencies of normal group were found to be higher than the scopolamine group, i.e., normal test group showed higher retention in memory (Graph 4). In case of water Rivastigmine test group and water-PHPE test group, test latencies of latter group were found to be slightly higher retention in memory (fig 3). In case of Scopolamine-Rivastigmine tst group and Scopolamine-PHPE test group, test latencies of latter group were found to be slightly higher i.e., scopolamine-PHPE test group showed slightly higher retention in memory (Graph 4).



Graph:4 Latency periods of graphs showing the latency period of all the groups in the test solution. Significant difference (P<0.05) was found to occur at SRTE and SPTE-I.

S.NO	GROUPS	LATENCY		MEAN	MEAN±SEM
		TRAIL I	TRAIL II		
1	NTE	44	44	44	44±1.92
		36	36		
		46	46		
		50	50		
2	STE	30	30	24.5	24.5±1.39
		27	27		
		19	19		
		22	22		
3	RTE	12	12	25.25	25.25±3.26
		28	28		
		25	25		
		36	36		
4	PTE-I (200mg/kg)	27	27	26.5	26.5±
		39	39		
		22	22		
		18	18		
5	PTE-II (133mg/kg)	38	38	37.25	37.25±0.49
		36	36		
		39	39		
		36	36		
6	PTE-III (80mg/kg)	46	46	42.75	42.75±0.81
		43	43		
		40	40		
		42	42		
7	SRTE	38	38	37.25	37.25±0.49
		36	36		
		39	39		
		36	36		
8	SPTE-I (200mg/kg)	22	22	30.25	30.25±0.49
		26	26		

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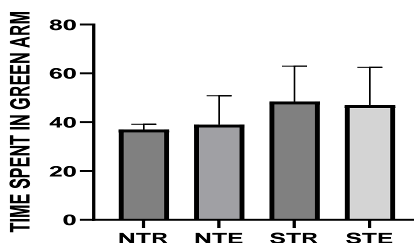
		32	32		
		42	42		
9	SPTE-II (133mg/kg)	24	24	25.75	25.75±1.52
		31	31		
		29	29		
		19	19		
10	SPTE-III (80mg/kg)	16	16	23	23±3.60
		12	12		
		28	28		
		36	36		

Table 10: The Latency periods of test sessions of all groups were assessed. The average latency periods (rounded off to nearest whole number) and SEM was calculated.

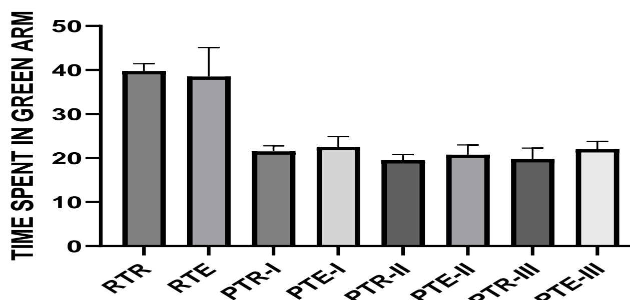
5.4 T MAZE TEST

Zebra fish were also assessed in the T-maze containing coloured plastic sheets fitted around the three sides of the short arms of the T maze (fig 4). The number of entries and time spent in each compartment (Figure A) were recorded. The comparisons of time spent in the green arm of normal group and scopolamine group showed that there is a reduction in memory in case of scopolamine group. Interestingly, the time spent by scopolamine treated group is less than the time spent in the green arm by the normal group.



Graph:5 Graph showing the time spent (sec) in green arm (Feeding chamber) of normal (water only) scopolamine treated groups in training and test sessions.

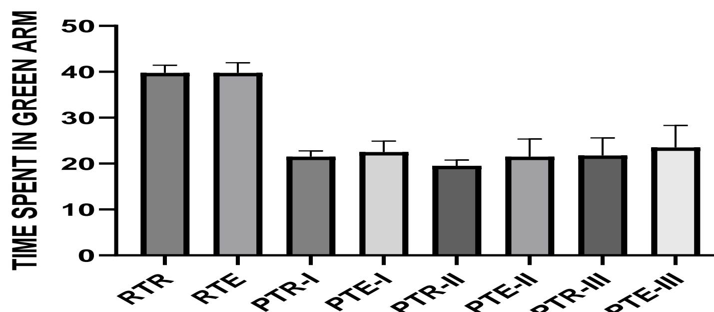
The effectiveness of the poly herbal plant extracts was evaluated by combining the PHPE extract with the scopolamine exposure before training and test sessions. The time spent in the green arm of training and test group of normal and scopolamine groups of test poly herbal plant extracts were compared with the time spent in the green arm of normal and scopolamine groups of standard (RIVASTIGMINE) group (graph 6 and 7). The comparison showed that the time spent in the green arm of PHPE-I groups is slightly less than compared to Rivastigmine groups. Similarly the time spent in the green arm of PHPE-II (PTE-II) and PHPE-III (PTE-III) is slightly compared to PHPE-I.



Graph: 6 Graph showing the time spent (sec) in the Green arm (Feeding chamber) of Normal Rivastigmine groups and Normal PHPE I, PHPE II, and PHPE III groups in training and test sessions.

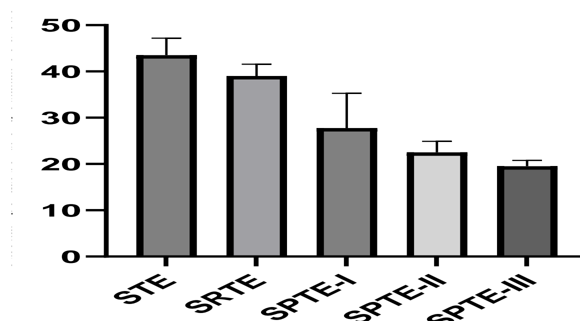
S.NO	GROUPS	TIME SPENT	MEAN	MEAN±SEM
1	NTR	38	36.5	36.5±3.92
		42		
		25		
		41		
2	NTE	40	33.25	33.25±4.92
		24		
		29		
		40		
3	STR	42	45.25	45.25±6.36
		36		
		64		
		39		
4	STE	60	45.5	45.5±7.42
		51		
		46		
		25		

Table 11: The time spent in the green arm (seconds) of normal (water only) and only scopolamine groups were assessed. The average and SEM of time spent in the green arm was calculated.



Graph: 7 Graph showing the time spent in Green arm scopolamine treated Rivastigmine group and scopolamine treated PHPE I, PHPE II, and PHPE III groups in training and test sessions.

The time spent in green arm i.e., retention of memory in case of scopolamine PHPE-I (PTE-I) group is higher than the only scopolamine treated group but this is slightly lesser in case of Rivastigmine group than scopolamine treated PHPE-I (PTE-I) group. The trend had been followed in case of PHPE-II (PTE-II) and PHPE-III (PTE-III) groups.



Graph: 8 Graph showing the time spent (sec) in the Green arm (Feeding chamber) of all the groups in the training and test session. Significant difference (P,0.05) was found to occur at SRTE and SPTE-I.

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Table 12: The time spent in the green arm (seconds) of normal Rivastigmine group and normal PHPE I, PHPE II, and PHPE III

S.NO	GROUPS	TIME SPENT	MEAN	MEAN±SEM
1	RTR	43	32	32±6.12
		45		
		40		
		41		
2	RTE	46	20.25	20.25±0.62
		43		
		40		
		42		
3	PTR-1 (200mg/kg)	38	41.25	41.25±2.92
		36		
		39		
		36		
4	PTE-I (200mg/kg)	43	41.25	41.25±5.35
		45		
		40		
		41		
5	PTR-II (133mg/kg)	46	51.75	51.75±4.16
		43		
		40		
		42		
6	PTE-II (133mg/kg)	35	34	34±9.06
		37		
		40		
		36		
7	PTR-III (80mg/kg)	38	44	44±2.34
		36		
		39		
		36		
8	PTE-III (80mg/kg)	44	47.75	47.75±5.94
		36		
		46		
		50		

S.NO	GROUPS	TIME SPENT	MEAN	MEAN±*SEM*
1	SRTR	43	42.25	±1.10
		45		
		40		
		41		
2	SRTE	46	42.75	±1.25
		43		
		40		
		42		
3	SPTR-1 (200mg/kg)	38	37.25	±0.75
		36		
		39		
		36		
4	SPTE-I (200mg/kg)	43	42.25	*42.25*±1.10
		45		
		40		
		41		
5	SPTR-II (133mg/kg)	46	42.75	*42.75*±1.25
		43		
		40		
		42		
6	SPTE-II (133mg/kg)	35	37	*37*±1.08
		37		
		40		
		36		
7	SPTR-III (80mg/kg)	38	37.25	*37.25*±0.75
		36		
		39		
		36		
8	SPTE-III (80mg/kg)	44	44	*44*±2.34
		36		
		46		
		50		

Table 13: The time spent in the green arm scopolamine treated Rivastigmine group groups were assessed.

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The average and SEM of time spent in the green arm was calculated.and scopolamine treated PHPE I, PHPE II, and PHPE III groups in training and test sessions. and scopolamine treated PHPE I, PHPE II, and PHPE III groups in training and test sessions.

S.NO	GROUPS	TIME SPENT	MEAN	MEAN±SEM
1	NTE	38	37.25	±0.75
		36		
		39		
		36		
2	STE	46	42.75	±1.25
		43		
		40		
		42		
3	RTE	35	37	37±1.08
		37		
		40		
		36		
4	PTE-I (200mg/kg)	38	37.25	37.25±0.75
		36		
		39		
		36		
5	PTE-II (133mg/kg)	43	42.25	42.25±1.10
		45		
		40		
		41		
6	PTE-III (80mg/kg)	12	25.25	25.25±2.49
		28		
		25		
		36		
7	SRTE	27	26.5	26.5±4.55
		39		
		22		
		18		
8	SPTE-I (200mg/kg)	19	27.25	27.25±7.04
		12		
		42		
		36		
9	SPTE-II (133mg/kg)	44	44	44±2.34
		36		
		46		
		50		
10	SPTE-III (80mg/kg)	34	32.25	32.25±3.75
		25		
		28		
		42		

Table 14: The time spent in the green arm (seconds) of Test sessions of all groups were assessed. The average and SEM of time spent in the green arm was calculated.

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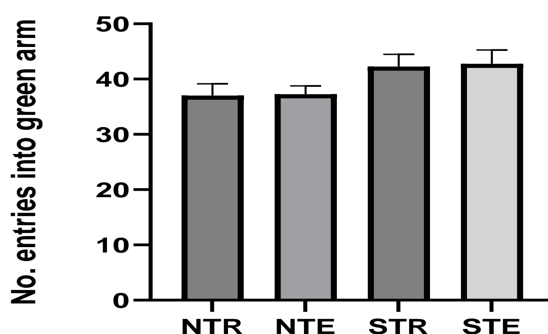
No. of entries into green arm in case of water and scopolamine treated Rivastigmine and groups showed that there is a decrease in no. of entries in the test sessions compared to training sessions i.e., there is a retention of memory by scopolamine treated Rivastigmine and PHPE I (PTE-I) groups compared to compared to normal (water) and scopolamine (only) groups but scopolamine treated Rivastigmine group has more memory retention than scopolamine treated PHPE I groups (Fig 9, 10 and 11). Similarly PHPE-II and PHPE-III has less memory retenti0on compared to PHPE I.



Graph: 9 Graph showing No. of entries into the green arm (Feeding chamber) of Normal water only) and only scopolamine treated groups in the training and test sessions.

S.NO	GROUPS	NO.OF ENTRIES	MEAN	MEAN±SEM
1	NTR	35	37	37±1.08
		37		
		40		
		36		
2	NTE	38	37.25	37.25±0.75
		36		
		39		
		36		
3	STR	43	42.25	42.25±1.10
		45		
		40		
		41		
4	STE	46	42.75	42.75±1.25
		43		
		40		
		42		

Table 15: TheNo. of entries into the green arm of normal (water only) and only scopolamine groups were assessed. The average and SEM of time spent in the green arm was calculated.

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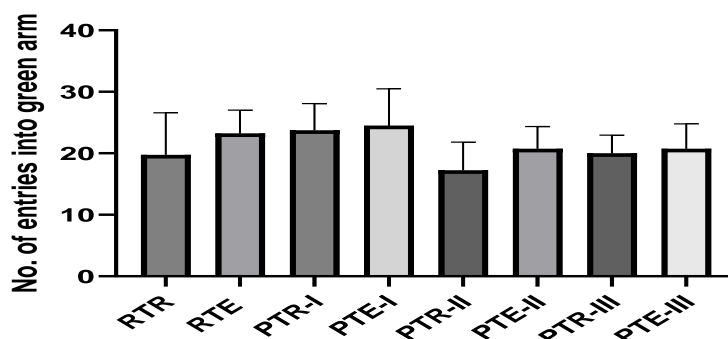
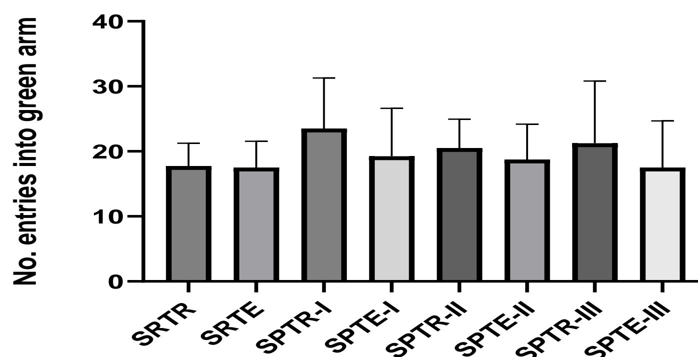


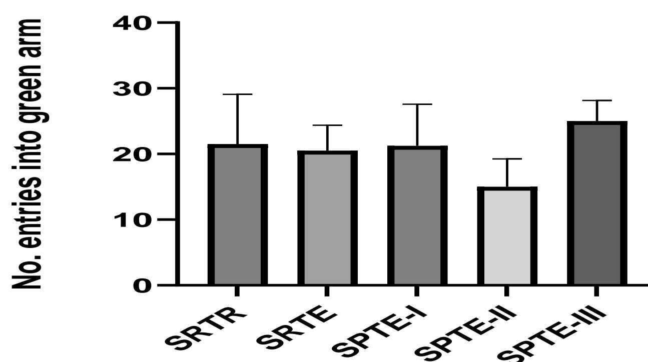
Graph: 10 Graph showing No. of entries into the green arm (Feeding chamber) of Normal Rivastigmine and PHPE I, PHPE II, and PHPE III groups in training and test sessions.

S.NO	GROUPS	NO. OF ENTRIES	MEAN	MEAN±SEM
1	RTR	12	19.75	19.75±3.42
		25		
		16		
		26		
2	RTE	19	23.25	23.35±1.88
		22		
		28		
		24		
3	PTR-I (200mg/kg)	23	23.75	23.75±2.17
		20		
		22		
		30		
4	PTE-I (200mg/kg)	18	24.5	24.5±2.98
		22		
		26		
		32		
5	PTR-II (133mg/kg)	12	17.25	17.25±2.28
		18		
		23		
		16		
6	PTE-II (133mg/kg)	19	20.75	20.75±1.79
		26		
		20		
		18		
7	PTR-III (80mg/kg)	21	20	20±1.47
		20		
		16		
		23		
8	PTE-III (80mg/kg)	23	20.75	20.75±1.79
		16		
		19		
		25		

Table 16: The No. of entries in green arm of normal Rivastigmine and PHPE I, PHPE II, and PHPE III groups were assessed. The average and SEM of time spent in the green arm was calculated.



Graph: 11 Graphs showing No. of entries in green arm of scopolamine treated Rivastigmine group and PHPE I, PHPE II, and PHPE III groups in training and test sessions.



Graph: 12 Graphs showing No. of entries into the green arm (Feeding chamber) of all the groups in the test session.

S.NO	GROUPS	NO.OF ENTRIES	MEAN	MEAN±SEM
1	SRTR	14	17.75	17.75±1.75
		19		
		16		
		22		
2	SRTE	20	17.5	17.5±2.02
		17		
		21		
		12		
3	SPTR-1 (200mg/kg)	21	23.5	23.5±3.88
		14		
		27		
		32		
4	SPTE-I (200mg/kg)	28	19.25	19.25±3.68
		22		
		16		
		11		
	SPTR-II (133mg/kg)	22	20.5	20.5±2.21
		26		

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5		16		
		18		
6	SPTE-II (133mg/kg)	26	18.75	18.25±2.71
		19		
		13		
		17		
7	SPTR-III (80mg/kg)	14	21.25	21.25±4.78
		12		
		29		
		30		
8	SPTE-III (80mg/kg)	19	17.5	17.5±2.02
		13		
		27		
		11		

Table 17: The No. of entries in green arm of scopolamine treated Rivastigmine group and PHPE I, PHPE II, and PHPE III groups in training and test sessions.

S.NO	GROUPS	NO.OF ENTRIES	MEAN	MEAN±SEM
1	NTE	32	21.5	21.5 ±3.79
		21		
		14		
		19		
2	STE	25	20.5	20.5±2.21
		19		
		16		
		22		
3	RTR	12	21.25	21.25±5.39
		24		
		36		
		15		
4	PTE-I (200mg/kg)	14	21.25	21.25±4.78
		19		
		23		
		29		
5	PTE-II (133mg/kg)	18	30.75	30.75±5.02
		29		
		34		
		42		
6	PTE-III (80mg/kg)	20	15	15±2.12
		12		
		17		
		11		
7	SRTE	17	26.5	26.5±4.55
		27		
		29		
		33		

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8	SPTE-I (200mg/kg)	28	25	25±1.15
		27		
		24		
		21		
9	SPTE-II (133mg/kg)	25	29.5	29.5±4.73
		20		
		31		
		42		
10	SPTE-III (80mg/kg)	46	37.5	37.5±3.77
		28		
		36		
		40		

Table 18: The No. of entries into the green arm of the test session of all the groups was assessed. The average and SEM of time in the green arm was calculated.

5.5 DISCUSSION

In the present study, we have evaluated the effect of poly herbal plant extract with different doses by conducting behavioural tests namely Inhibitory avoidance Test and T-maze test. Poly herbal plant extract-I has shown more memory enhancing activity compared to PHPE-II and PHPE-III. Scopolamine, an anti-cholinesterase drug was used as amnesic agent in our study though it can be used as a drug of choice for motion sickness, shaking palsy and opioid addiction. Our results have shown that scopolamine administration had study cognitive impairment.

The test drug, poly herbal plant extract-1 acts as acetylcholinesterase inhibitor, anti oxidative.Anti-inflammatory, Therefore the standard drug that was chosen for our study was Rivastigmine which is an Acetylcholinesterase inhibitor currently present in the market. prescribed for the patients suffering from Alzheimer's disease. The test drug (poly herbal plant extract-1) results were then compared with the standard (Rivastigmine) and only scopolamine and normal groups.

Passive Avoidance Test (PAT) is an aversive conditioning task, have been shown useful to analyse cholinergic effects on memory. Our results of PAT have shown that scopolamine fairly induced cognitive impairment. But the administration of PHPE I (200mg/kg) and Rivastigmine (1.5mg/kg) reduced the memory impairment. The parameter that was evaluated in PAT was latency period. The latency period of only scopolamine group was very less compared to the standard and test groups. The latency periods of test drug (PHPE I) were compared with that of the standard, Normal and only scopolamine treated groups.

Zebra fishes were also assessed for memory using T-maze test. We designed this test based on the work carried out by Avdesh et.al. They evaluated colour preference of zebra fish for learning and memory using four different colours viz., blue, green, and red, yellow. Among the four colours zebra fish has shown equal preference to green and red. Therefore, we used green and red colour for our study to avoid the colour bias. In this test, green colour arm has been designated as a food arm where zebra fish was awarded food. We trained all the groups by awarding food in the green arm for 6 days and test session was conducted on the next day i.e. on 7th day. The results of last day of training were considered as the training session results.

Comparison of test session results were done with the training session results in thegroups and among the groups.

The parameters evaluated in T maze were Time spent in the green arm and No. of entries into green arm. The only scopolamine treated group spent less time in the green arm compared to the scopolamine treated standard and test groups. Time spent by the standard group was slightly higher than the test group. No. of entries into the green arm was found to be less in case of standard and test groups compared to scopolamine group.

5.6 POSSIBLE MECHANISM OF ACTION

In the present study, Scopolamine is used as an inducing agent of Alzheimer's disease, which is a Muscarinic Antagonist, also known as Anti-cholinergic drug. It binds to the muscarinic receptor and blocks the actions of acetylcholine such as release of Inositol Triphosphate (IP_1) and inhibition of Adenyl Cyclase. Poly herbal plant extract-1 which was used as test drug in the present study is an anti-Alzheimer's drug, which inhibits

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the enzyme Acetylcholinesterase that breaks down the Acetylcholine in vivo, Due to the Neurodegeneration in the people with Alzheimer's Disease the production of Acetylcholine is declined. So, the poly herbal plant extract-1 which acts as cholinesterase inhibitor, antioxidative and anti-inflammatory, enables the availability of Acetylcholine at the receptor site so that competitive blockade of the muscarinic receptor by scopolamine can be prevented. This might be the possible Mechanism of Action of poly herbal plant extract.

VI. CONCLUSION

Till now, drugs that were approved and currently running in the market can only manage the symptoms. So, there is an urgent requirement for finding the treatment that cures the disease. Alzheimer's is multifactorial disorder, the medication is used to treat the disease should have four way mechanism. Most of the synthetic drugs have side effects and interactions So, it would be better to develop the drugs from the plant origin because these drugs doesn't show side effect, good compatibility and also which would be rather economical. Based on the results obtained the Poly herbal plant extract-1 can be used as a potential drug for the Alzheimer's disease.

VII. SUMMARY

Alzheimer's disease is a neurodegenerative disorder which is characterized by the progressive loss of memory and can also include motor disability, hallucinations, speech impairment, depression, delusion and progressive behavioural disturbances through the disease course. It is the most common cause of dementia in the people of aged 60 and above and India accounts for about 3.4% of the global prevalence. There are certain drugs in the markets prescribed for the patients with Alzheimer's disease such as Rivastigmine, Donepezil, Memantine etc., but there are some drawbacks for these drugs like side effects, acting by only one mechanism. Therefore, encouraging the Siddha and Ayurveda drugs is to be implemented for preventing the side effects and to treat the disease by affecting the multiple targets. In the present study, poly herbal plant extract which belongs to different families has been used as a test drug although the individual drugs already proved for the memory enhancement activity but the combination is not done. The zebra fish physiology resembles with the human beings better than the rodents, the attempt has been made to evaluate its activity in the zebra fish models namely Passive Avoidance Test and T maze test. The standard drug used in the study was Rivastigmine. The animals that were treated with (poly herbal plant extract) test drug, (Rivastigmine) Standard and scopolamine were evaluated. The results obtained showed that there was an enhancement in the memory in the animals treated with the poly herbal plant extract-I when compared to the scopolamine treated animals.

REFERENCES

[1]. AK Singh*, A Gupta2, AK Mishra2, V Gupta2, P Bansal, S Kumar Medicinal Plant for Curing Alzheimer’s Disease IJPBA 2010; 1(2):108-114

[2]. Alvarez XA, Cacabelos R, Laredo M, Couceiro V, Sampedro C, Varela M, et al; A 24 week, double-blind, placebo-controlled study of three dosages of Cerebrosysin in patients with mild to moderate Alzheimer’s disease; EUR J Neurol, 13, 2006, 43-54.

[3]. Avdesh, Mathew T Martin-Iverson, AlindaMondal, Mengqi Chen, SretenAskraba, Newman Morgan, MichealLardelli, David M Groth, Giuseppe Verdile and Ralph N Martins; Evaluation of colour preference in zebrafish for learning and memory; Proceedings of Measuring Behaviour,2010.

[4]. AzzaAbdelmageedMatloub et al., Optimization of polyphenol extraction fromVitis vinifera L. leaves, antioxidant activity and its correlation with ameliorationeffect on AlCl3-induced Alzheimer’s disease; Articles of Pharmaceutical sciencesAin university; 2018.

[5]. A.E.Bawaziretal.,EffectofBlackRaisins(Vitisvinifera)onAluminumChlorideInduced Alzheimer's Disease in Male Albino Rat; Effect of Black Raisins (Vitisvinifera)onAluminumChlorideInducedAlzheimer'sDiseaseinMaleAlbinoRat;2019.

[6]. Bachurin SO, Shevtsova EP, Kireeva EG, Oxenkrug GF, Sablin SO; Mitiochondria as a target for neurotoxins and neuroprotective agents; Ann NY AcadSci, 2003.

[7]. Barlow GW; the cichlid fishes: Nature’s grand experiment in evolution. New York: DaCapo Press: 2002.

[8]. Behrend ER, Bitterman ME, Sidman: Avoidance in the fish: Further studies of the CS-US interval; American journal of Psychology,77(1)m, 1964, 15-28.
[9]. Behrend ER, Bitterman ME; Avoidance-conditioning in the fish: Further studies of the CS-US internal; American Journal of Psychology, 77(1), 1964, 15-28.
[10]. Beis, D. & Stainer, D. Y. R. (2006). In vivo cell biology: Following the zebrafish trend. TRENDS in Cell Biology, 16, 105-112.
[11]. Braithwaite VA, Armstrong JD, McAdam HM, Huntingford FA: CAN juvenile Atlantic salmon use multiple cue systems in spatial learning? Animal Behaviour, 51(6), 1996, 1409.
[12]. Buckingham SD, Jones AK, Brown LA, Sattelle DB; Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection; Pharmacol Rev, 61, 2009, 39-61.
[13]. Chaturvedi RK, Beal MF; Mitochondrial approaches for neuroprotection; Ann NY AcadSci, 1147, 2008, 2008, 395-412.
[14]. ChengxuanQiu, Miia Kivipelto, Eva von Strauss; Epidemiology of Alzheimer's disease: occurance, determinants, and strategies toward intervention; Dialogues in Clinical Neuro science-Vol 11. No. 2. 2009.
[15]. Chen XQ Fawecett JR, Rahman YE, Ala TA, Frey IW; Delivery of nerve growth factor to the brain via the olfactory pathway; J Alzheimer'ws Dis, 1, 1998, 35-44.
[16]. Colwill RM, Raymond MP, Ferrerira L, Escudero H; Visual discrimination learning in zebrafish (Denio rerio); Behavioural Processes, 70(1), 2005,19-31.
[17]. David J. A novel behavioral test battery to assess global drug effects using the Zebrafish. IJCP, 2008, 21, 19-34.
[18]. D Anna Lindstrand et al., Zebrafish Models of Neurodevelopmental Disorders:Limitations and Benefits of Current Tools and Techniques; International Journalof Molecular Sciences; 14 March 2019.
[19]. Deepthi rapaka et al., Vitis vinifera acts as anti-Alzheimer's agent by modulatingbiochemicalparametersimplicatedincognitionandmemory;JournalofAyurvedaand Integrative Medicine; October–December 2019.
[20]. Drew MR, Zupan B, Cooke A, Couvillon PA, Balsam PD; Temporal control of conditioned responding in goldfish; Journal of Experimental Psychology: Animal Behavior Processes, 31(1), 2005, 31-39.
[21]. Driever, W., Solnica-Krezel, L., Schier, A. F., Neuhauss, S. C. F., Malicki, J., Stemple, D. L., Stainier, D. Y. R., Zwartkruis, F., Abdelilah, S., Rangini, Z., Belak, J., & Boggs, C. (1996). A genetic screen for mutations affecting embryogenesis in zebrafish. Development, 123, 37-46
[22]. Doody RS; we should not distinguish between symptomatic and disease-modifying treatments in Alzheimer's disease drug development; Alzheimer's Dement, 4, 2008, S21-5.
[23]. Eriksdotter-Jonhagen M. Nordberg A. Amberla K. Bäckman 1, Ebendal T. Meyerson B, et al, Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease; Dement GeriatrCognDisord, 9, 1998, 246-57.
[24]. Ellen J. Hoffman1,2 et al., Zebrafish Models of Neurodevelopmental Disorders:frontiersinMolecularNeuroscience;29August 2018.
[25]. Fetcho JR, Liu KS; Zebrafish as a model system for studying neuronal circuits and behaviour, Annals of the New York Academy of Sciences, 860, 1998, 333-345.
[26]. Grunwald, D. J. & Eisen, J. S. (2002). Headwaters of the zebrafish: Emergence of a new model vertebrate. Nature Reviews Genetics.
[27]. Ginpreetkauretal.,TheBrillianceoftheZebrafishModel:PerceptiononBehaviour and Alzheimer's Disease; frontiers journal; 13 June 2022.
[28]. Guo, S. (2004). Linking genes to brain, behaviour, and neurological diseases: What can we learn from zebrafish? Genes, Brain, and Behavior, 3, 63-74.
[29]. Haffter, P., Granato, M., Brand, M., Mullins, M. C., Hammerschmidt, M., Kane, D. A., Odenthal, J., van Eeden, F. J. M., Jiang, Y. J., Heisenberg, C. P., Kelsh, R. N., Furutani Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C.,

&Nüsslein-Volhard, C. (1996). The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. Development, 123, 1- 36.

[30]. Hajiaghaee R, Akhondzadeh S. Herbal Medicine in the Treatment of Alzheimer's Disease Journal of Medicinal Plants Vol.11no.41 2012

[31]. Higa JJ, Simm LA; Interval timing in Siamese fighting fish (Bettasplendens). Behavioural Processes, 67, 2004, 501-509.

[32]. Hollis KL; The role of learning in the aggressive and reproductive behaviour of blue georamas, Trichogastertrichopterus; Environmental Biology of Fishes, 54(4), 1999, 355-369.

[33]. Jakob-Roetne R, Jacobsen H; Alzheimer's disease: From pathology to therapeutic approaches; AngewChemint Ed, 48, 2009, 3030-59.

[34]. JintanapornWattanathornetal.,MoringaoleiferaMitigatesMemoryImpairmentandNeurodegenerationinAnimalModelofAge-RelatedDementia;PMCID;2013December 23.

[35]. Kihara T, Shimohama S, Urushitani M, Sawada H, Kimura J, Kume T, et al; Stimulation of alpha-4-beta-2 nicotinic acetylcholine receptors inhibits beta-amyloid toxicity, Brain Res, 792, 1998, 331-4.

[36]. Kanza M. Khan et al., Zebrafish Models in Neuro Psychopharmacology and CNSDrugDiscovery;TheUniversityofSouthernMississippitheAquilaDigitalCommunity; 7 July 2017.

[37]. KanathipSingsai et al., Research Article Effect of Streblus asper Leaf Extract onScopolamine-Induced Memory Deficits in Zebrafish: The Model of Alzheimer'sDisease; Hindawi Advances in Pharmacological and Pharmaceutical Sciences; 26April 2021.

[38]. Karthik Maddula et al., Assessment of Aqueous Extract of Ocimum sanctumLeaves in Memory Enhancement and Preventing Memory Impairment Activitiesin Zebra Fish Model; 2017 Journal of Basic and Clinical Pharmacy; 4 December2019.

[39]. Martina Blank, Laura D. Goerim, Reinaldo F. Condes, Monica RM. VA one-trial inhibitory avoidance task to zebrafish Rapidoption of NMDA dependent long-term memory, Neurobiology of Leaning and Memory, 90, 2000, 529-534.

[40]. Md.Abdulannanetal.,ResearchPaperMoringaoleiferawithpromisingneuronalsurvivalandneuriteoutgrowthpromotingpotentials;JournalofEthnopharmacology; 27February2014.

[41]. Miklosi A, Andrew RI, Savage H, Behaviourallateralisation of the tetrapod type in the zebrafish (Brachydaniorerio), Physiology &Behavior, 63(1), 1997, 127 135.

[42]. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schrooler BE, Shaked GM, et al; Neuroprotecive effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease; Nat Med, 15, 2008, 331-7.

[43]. Nadia Soussi-Yanicostas et al., Zebrafish as a Model for Neurological Disorders.International Journal of Molecular Sciences, 2022, 23; 13 April 2022.

[44]. Peleshok J, Saragovi HU; Functional mimetics of neurotrophins and theirreceptors; BiochimSoc Trans, 12, 2006, 1015-28.

[45]. Penberthy WT, Shafizadeh E, Lin S; The zebrafish as a model for human disease; Frontiers in Bioscience, 7, 2002, D1439-D1453.

[46]. Price RD, Milne SA, Sharkey J, Matsuoka N; Advances in small molecules promoting neurotrophic function, PharmacolTher, 115, 2007, 292-306.

[47]. Reader SM, Kendal JR, Laland KN; Social learning of foraging sites and escape routes in wild Trinidadian guppies, Animal Behaviour, 66(4), 2003, 729-739.

[48]. Reebs S; Fish behaviour in the aquarium and in the wild, Ithaca, NY: Comstock Pub. Assoc.

[49]. RimiHazraetal.,EffectofMoringaolieferainexperimentalmodelofAlzheimer'sdisease; Role of Anti-oxidants; Annals of neurosciences; July 2005.

[50]. Rohan S, Sharma et al., Neurocognitive Effect of Nootropic Drug Brahmi (Bacopamonnieri)inAlzheimer'sDisease;AnnalsofneurosciencesMay122017.

[51]. Sabbagh MN, Shill HA; Latrepirdine, a potential novel treatment for Alzheimer's

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 799-829 www.ijprajournal.com ISSN: 2249-7781

disease and Huntington's chorea; CurrOpin Invest Drugs, 11, 2010, 80-91.
[52]. Shahnawaz Ahmed et al., Zebrafish as a comprehensive model of neurologicaldiseases;ArticleinInternationalJournalofScienceandResearchArchive·September 2023.
[53]. Shamsetal.,Thezebrafishasapromisingtoolformodellinghumanbraindisorders: A review based upon an IBNS Symposium; 19 July 2022.
[54]. Soraya Santana et al., Review Article Can zebrafish be used as animal model tostudy Alzheimer's disease? ISSN May 30, 2012.
[55]. SyamimaAbdulmanapetal.,Bacopamonnieri,aNeuroprotectiveLeadinAlzheimerDisease:AReviewonItsProperties,Mechanismsof Action,andPreclinical and Clinical Studies; sage journals; 31 july 2019.
[56]. SamirAydietal.,StudyontheChemicalCompositionandtheBiologicalActivitiesof Vitis vinifera Stem Extracts; molecules journals; May 2022.
[57]. SouadElGengaihietal.,GrapeSeedsExtractasBrainFood;InternationalJournalofPharmaceuticalandClinicalResearch2017;25thJanuary,2017.
[58]. UroojFatima et al., Investigatingneuroprotectiveroles of Bacopamonnieri extracts:Mechanisticinsightsandtherapeuticimplications;sciencedirectjournal;September2022.