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NEUROPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *BIXA ORELLANA* AGAINST SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN ANIMAL MODE.

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INTRODUCTION:

In today's stressful world, drugs with neuroprotective and neuropharmacological action that improve brain learning and memory function are desperately needed. Importantly, stress has been shown to impair cognitive abilities, causing the memory to deteriorate rather than learning to progress[1].

For researchers, mental diseases have long been a source of disinterest, and treating them has always been difficult. Dementia, or memory loss, has captivated the human population all over the world, and it has become a hot topic of research. Human memory is known to decline with age[2].

Science and medical technology have now made it possible to augment humans in a variety of ways. The possibility of improving our cognitive performance has recently become both innovative and substantial. There are currently several supplements available, such as caffeine for staying awake and DHA for improved memory.

However, the latest treatments for improving our cognitive abilities, known as smart pharmaceuticals, are nothing like these supplements. Smart medicines are man-made synthetic substances that have been produced to increase human cognitive abilities. They were originally designed for medicinal purposes, but they can also benefit healthy people. The study of how drugs might improve human intellect began in the nineteenth century[3].

Cognitive dysfunction is one of the most functionally destructive aspects of many neuropsychiatric and neurodegenerative illnesses, including schizophrenia, depression, Alzheimer's disease, dementia, cerebrovascular impairment, seizure disorders, head injury, and Parkinsonism. By impairing long-term potentiation (LTP) induction and synaptic plasticity, aging plays a significant role in the development of cognitive dysfunction such as age-related memory impairment (AAMI).

Our mental abilities are controlled by the brain, which is the organ in charge of them. All of our ideas, emotions, desires, perceptions, learning and memory, curiosity, and behavior are built on this basis. We are restricted to fundamental reflexes and stereotyped reactions without memory, which is a distinct mental activity.[4]

Alzheimer's disease is the primary cause of dementia in the elderly, affecting an estimated 15 million individuals globally. With the proportion of aged persons in the population steadily growing, the disease's impact on treatments and national economies is predicted to grow significantly over the next two to three decades[5].

The most significant risk factor for Alzheimer's disease is growing older. AD affects 7% of those aged 65 to 74, 53% of people aged 75 to 84, and 40% of people aged 85 and more. 3 Although other variables may play a role, genetic inheritance is a substantial risk factor. The factors that determine the age of start and the pace of progression are mostly unknown.

After the beginning of symptoms, survivors are expected to live for 3 to 20 years, with an average of 8 years. Individuals with Alzheimer's disease live around half as long as others of the same age who do not have the disease. In the United States, Alzheimer's disease is the sixth greatest cause of mortality disease. Although Alzheimer's disease does not directly cause mortality, it does put patients at risk for sepsis, pneumonia, choking and aspiration, nutritional deficits, and trauma[6].

1. Learning and memory:

Learning is one of man's and higher animals' most distinguishing characteristics. Learning is defined as the ability to change behavior based on prior experience7. Memory is a particular feature of the brain that allows it to remember events that occur throughout the learning process, and both are mediated by the nervous system8. When memories are stored in the brain, they become part of the brain's processing machinery when it comes time to recall them in the future9. Learning and memory are inextricably intertwined; all learning necessitates memory, but not all memory necessitates learning10. Sensory information obtained through the eyes, hearing, and other senses is stored in short-term or iconic memory (Greek: Iconic = Image) for a relatively limited period.

Some information from the iconic memory lingers in short-term memory for a few seconds. 11. Declarative memory (also known as explicit memory) is the ability to recall information and experiences consciously. When the phrases "memory" or "remembering" are employed in everyday English, they normally relate to this type of memory. Nondeclarative (or implicit) memory, a heterogeneous set of nonconscious abilities that includes the learning of skills and habits, priming, and some types of typical conditioning, can be compared with declarative memory. Experience accumulates in behavioral change in these circumstances, but without allowing access to any memory information. Because it has been shown that different types of memory are maintained by different brain systems, the distinction between declarative and nondeclarative memory is crucial.

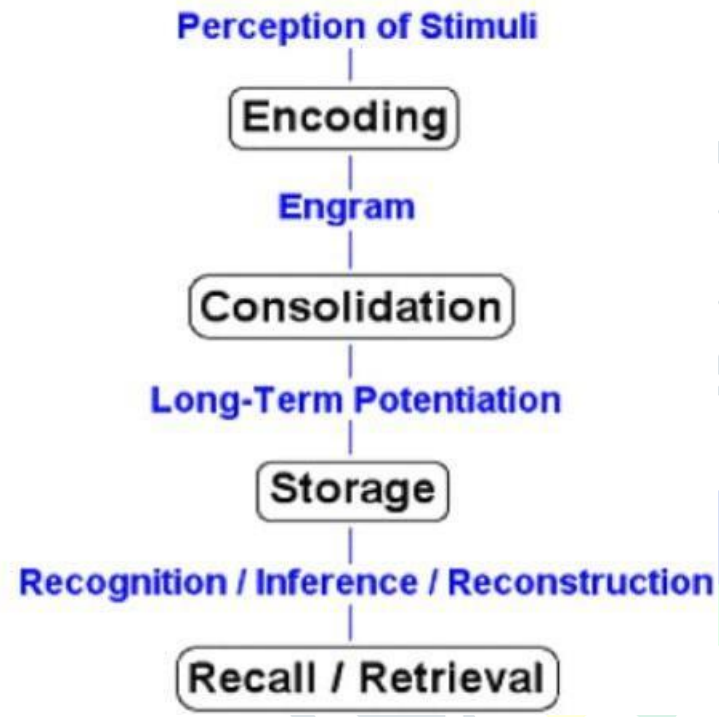

Fig. 1.2: Memory storage and its recall.

2. Types of Memory

Some memories endure only a few seconds, while others last for hours, days, months, or even a year. For this discussion, let us assume a popular memory classification system that divides memories into the following categories:

2.1. **Short-term memory:** Unless they are turned into longer-term memories, including recollections that endure seconds or minutes.

2.2.

2.3. **Intermediate long-term memories:** can continue for a few days to weeks before dissipating.

2.4. **Long-term memory:** which, if saved, can be retrieved years, if not a lifetime later.

2.5. **Working memory:** mostly short-term memory that is employed during intellectual thinking but is ended when each stage of the problem is addressed 9. Memories are usually divided into categories according to the sort of data they contain. Memory is divided into declarative and nondeclarative memory according to one of

these classifications:

Declarative memory: It involves fact and event memory and is dependent on the hippocampus and associated structures' integrity.

Non-Declarative memory: It refers to a heterogeneous collection of unique learning and memory capacities in which the component changes but access to the events that generated the change is denied. 12.

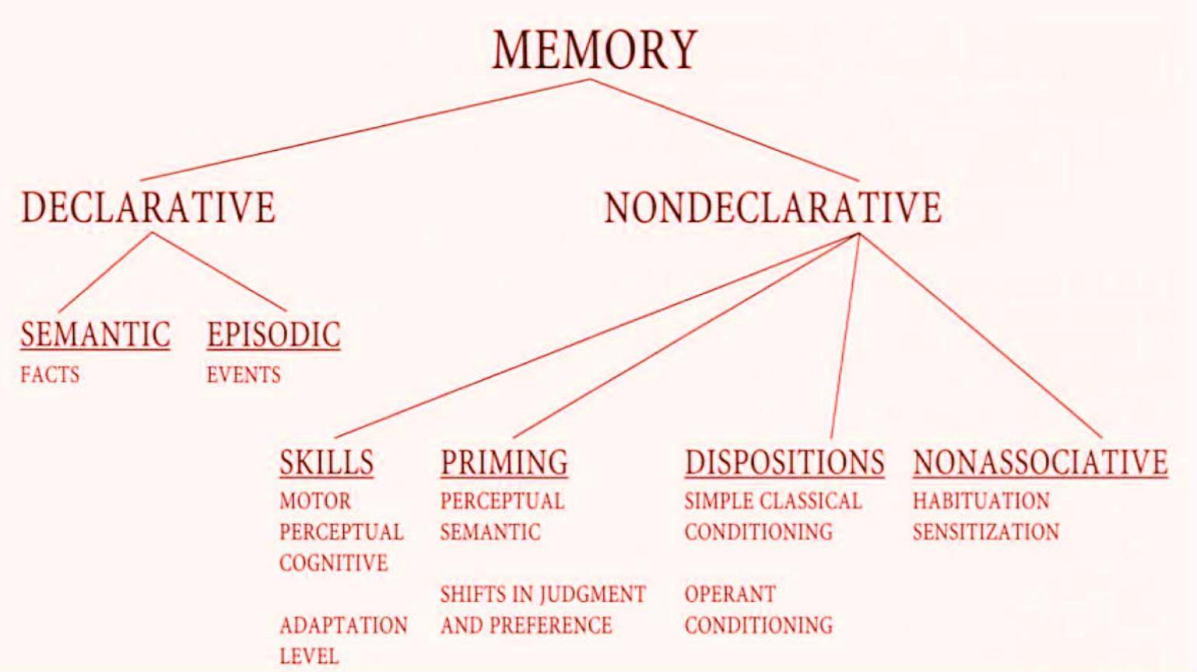

Fig. 1.3: Types of memory

Dementia:

Dementia is defined as the loss of sufficient logical ability (medically referred to as cognitive impairment) to interfere with social or occupational functioning13. It can be caused by a variety of disorders that harm brain cells. There are several varieties of dementia, each with its unique set of symptoms and causes. Long-term epidemiological observation and postmortem investigations are increasingly indicating that many persons have brain abnormalities linked to more than one kind of dementia14.

2.6. Types of Dementia:

2.6.1.Alzheimer's disease:

The most prevalent kind of dementia, accounting for 60 to 80 percent of cases. Apathy and despair are also common early clinical signs, as are difficulty recalling names and recent events. Impaired judgment, disorientation, confusion, behavioral abnormalities, and difficulties speaking, swallowing, and walking are some of the later signs. In 2011, new diagnostic criteria and recommendations for Alzheimer's disease were proposed. They urge that Alzheimer's disease be treated as a disease that begins long before symptoms appear.

2.6.2.Vascular dementia:

Vascular dementia, also known as multi-infarct or post-stroke dementia, is a less prevalent form of dementia than Alzheimer's disease. Rather than memory loss, which is generally connected with the first symptoms of Alzheimer's, impaired judgment or the ability to make plans is more likely to be the first sign. Brain traumas such as microscopic hemorrhage and blood artery obstruction induce vascular dementia. The impact of a brain injury on an individual's mental and physical functioning is determined by the location of the lesion.

Evidence of vascular dementia was once used to rule out an Alzheimer's disease diagnosis (and vice versa). This method is no longer regarded as compatible with pathologic research, which reveals that both kinds of dementia can appear with brain abnormalities at the same time. A person is said to have "mixed dementia" when two or more kinds of dementia are present at the same time.

1.2.52 Dementia with Lewy bodies (DLB):

People with DLB exhibit some of the same symptoms as persons with Alzheimer's, but they are more likely to experience beginning or early symptoms including sleep problems, well-formed visual hallucinations, and muscular stiffness or other parkinsonian movement characteristics than people with Alzheimer's. Altered aggregations (or clumps) of the protein alpha-synuclein are known as Lewy bodies.

Dementia can occur when they form in the cortex, a region of the brain. People with Parkinson's disease have alpha-amyloid aggregates in their brains, however, the aggregates may not form in the same way as DLB. DLB's brain alterations can induce dementia on their own, or they can occur in conjunction with Alzheimer's disease and/or vascular dementia, with each contributing to the development of dementia. When this happens, the person is diagnosed with "mixed dementia."

2.6.3.Frontotemporal lobar degeneration (FTLD):

Dementias such as behavioral variant FTLD, primary progressive aphasia, Pick's disease, and progressive supranuclear palsy are all included.

Changes in personality and conduct, as well as language difficulties, are common signs. Nerve cells in the front and sides of the brain are particularly vulnerable. There is no one microscopic anomaly that is connected to all of the instances. Although the brain alterations of behavioral variant FTLD and Alzheimer's disease may occur at the same time, persons with behavioral variant FTLD acquire symptoms at a younger age (about age 60) and live for fewer years than those with Alzheimer's.

2.6.4.Mixed dementia:

The characteristic anomalies of Alzheimer's disease and another kind of dementia, most often vascular dementia, but sometimes other types, such as DLB, are present. Mixed dementia appears to be more widespread than previously assumed, according to recent research.

2.6.5.Parkinson's disease:

Parkinson's disease typically leads to severe dementia that resembles DLB or Alzheimer's disease as it advances. Early on in the condition, problems with mobility are a typical sign. The substantia nigra, a deep part of the brain, is where alpha-synuclein aggregates are most prone to develop. The aggregates are hypothesized to induce dopamine-producing nerve cells to degenerate. Parkinson's disease is roughly one-tenth as common as Alzheimer's disease.

2.6.6.Creutzfeldt- Jakob disease:

A rapidly deadly illness that produces behavioral abnormalities and affects memory and coordination. The disease is caused by an infectious misfolded protein (prion) that causes other proteins in the brain to misfold and malfunction. Variant Consumption of products from animals infected with mad cow disease is thought to be the cause of Creutzfeldt-Jakob disease

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2.6.7.Normal pressure hydrocephalus:

The accumulation of fluid in the brain causes symptoms such as trouble walking, memory loss, and inability to regulate urine. It is occasionally possible to treat this condition by surgically implanting a shunt in the brain to drain excess fluid. [15]

3. Alzheimer's disease:

The most frequent kind of dementia is Alzheimer's disease (AD). The condition has no treatment and worsens as it continues, finally leading to death. It was named after German psychiatrist and neuropathologist Alois Alzheimer, who originally characterized it in 1906. Alzheimer's disease is a central nervous system degenerative disease. Dementia, behavioral, and cognitive deficits will eventually result from the progression of diseases. Selective neuronal death, the development of extracellular amyloid deposits in the core of neuritic plaques, and the production of intraneuronal neurofibrillary tangles in afflicted people's brains are all symptoms of Alzheimer's disease. Alzheimer's disease affects up to 15% of persons over the age of 65, and almost half of those over the age of 85. The significant loss of cholinergic neurons in the nucleus basalis and adjacent regions that make up the cholinergic forebrain area, as well as their protrusion into the cerebral cortices, is noticeable in Alzheimer's disease. It's not always easy to tell Alzheimer's disease from other types of dementia. Alzheimer's disease is characterized by increasing memory, judgment, decision-making, orientation to a physical environment, and language dysfunction. The history of symptoms and a neurological examination are typically used to get a working diagnosis of Alzheimer's disease. However, a conclusive diagnosis can only be determined after death, when an autopsy is performed. Alzheimer's disease is characterized by unique helical protein filaments in the brain's neurons on a cellular level: Neurofibrillary tangles are a kind of neurofibrillary tangle.

Alzheimer's disease is a neurological illness that affects 22 million people globally, with over 3 million in India, according to the World Health Organization (WHO). Its frequency climbs dramatically from around 5% at 95 years old to about 20%. Despite the availability of medications that try to delay the progression of the disease, researchers have yet to find a definitive cure for Alzheimer's disease. 19 Nearly four decades ago, a Belgian neurophysiologist active in drug research proposed the notion of nootropic drugs20, which he defined as medicines that promote, improve, and protect cognitive performance. Smart medicines, memory enhancers, neuroenhancers, cognitive enhancers, and intelligence enhancers are pharmaceuticals, supplements, nutraceuticals, and functional foods that claim to boost mental processes including cognition, memory, intellect, motivation, attention, and concentration21. Dr. Corneliu E. Giurgea22 of Romania invented the term nootropic in 1972, derived from the Greek terms nous, which means "thought," and trepein, which means "to bend/turn."

3.1. History of AD:

Alois Alzheimer discovered Alzheimer's disease in 1907, but it was not recognized as a severe disease or problem until the 1970s23. Auguste D., a 51-year-old woman, was the first to be diagnosed with the condition. After seeing changes in her conduct and demeanor, her family brought her to Dr. Alzheimer in 1901. Memory issues, difficulties communicating, and a lack of comprehension were all mentioned by the family. Auguste was eventually diagnosed with a severe type of dementia, which manifested itself in memory, language, and behavioral deficits24, according to Dr. Alzheimer. Alois Alzheimer presented an exceptional presentation on November 4, 1906, in which he described for the first time a kind of dementia that would later be recognized as Alzheimer's disease. Alzheimer reported a patient named Auguste D, a 51-year-old lady from Frankfurt, who had increasing cognitive impairment, focal symptoms, hallucinations, delusion, and psychosocial incompetence in his speech at the 37th meeting of South-West German Psychiatrists in Tubingen. Plaques, neurofibrillary tangles, and arteriosclerotic alterations were discovered during necropsy. The term Alzheimer was first coined to describe presenile dementia, but it was eventually adopted to describe the most common form of primary dementia, senile dementia of the Alzheimer type 25. In 1907, the disorder was first mentioned in medical literature, and in 1910, it was given the term Alzheimer's disease.

3.2. Anatomy and physiology associated with dementia:

All dementias fall into one of two categories: cortical or subcortical brain degeneration. Memory warfare is a prominent feature of cortical dementias. AD is caused by cortical dysfunction, although it eventually affects all lobes to some extent26. As the condition advances, magnetic resonance imaging (MRI) has revealed that it appears to occur in distinct parts of the brain27. Motor difficulties are frequently related to subcortical abnormalities. Memory is a process that involves the entire brain. The hippocampus (placed deep in the brain

above the brain stem) and the amygdala (located under the temporal lobe) are thought to be important in memory development, storage, and retrieval. The hippocampus, which is related to sensory parts of the cortex via afferent pathways, is responsible for the acquisition and temporary storage of declarative memory. Declarative memory allows people to arrange their surroundings. For example, if someone learns the way to work and travels it a few times, they will be able to get there even if there is an alternate route. The hippocampus keeps track of all of these memories so they may be retrieved when they're needed. Individuals who have had simultaneous hippocampal loss can only record incoming stimuli until the next one comes. Memories can't be recalled when they're needed, as during a learning experience28. Acetylcholine is the neurotransmitter that plays the most important role in Alzheimer's disease. Dysfunction and reduction of nicotinic acetylcholine receptors are linked to adverse cognitive and neurodegenerative effects[29].

3.3. Pathophysiology of AD:

Neuritic plaques and neurofibrillary tangles (NFTs) in the cortical regions and medial temporal lobe structures of the brain are the hallmark lesions of Alzheimer's disease. Degeneration of neurons and synapses, as well as cortical atrophy, accompany these lesions. Plaques and NFTs can be seen in various illnesses and even in normal aging, but plaques and NFTs are found in considerably larger concentrations in Alzheimer's patients. The conditions under which these lesions lead to the clinical manifestations of Alzheimer's disease are unknown. AP aggregation and deposition leading to plaque formation; hyperphosphorylation of tau protein leading to NFT development; inflammatory processes; neuro vasculature and dysfunction; have all been hypothesized as pathways to explain these alterations in the brain.

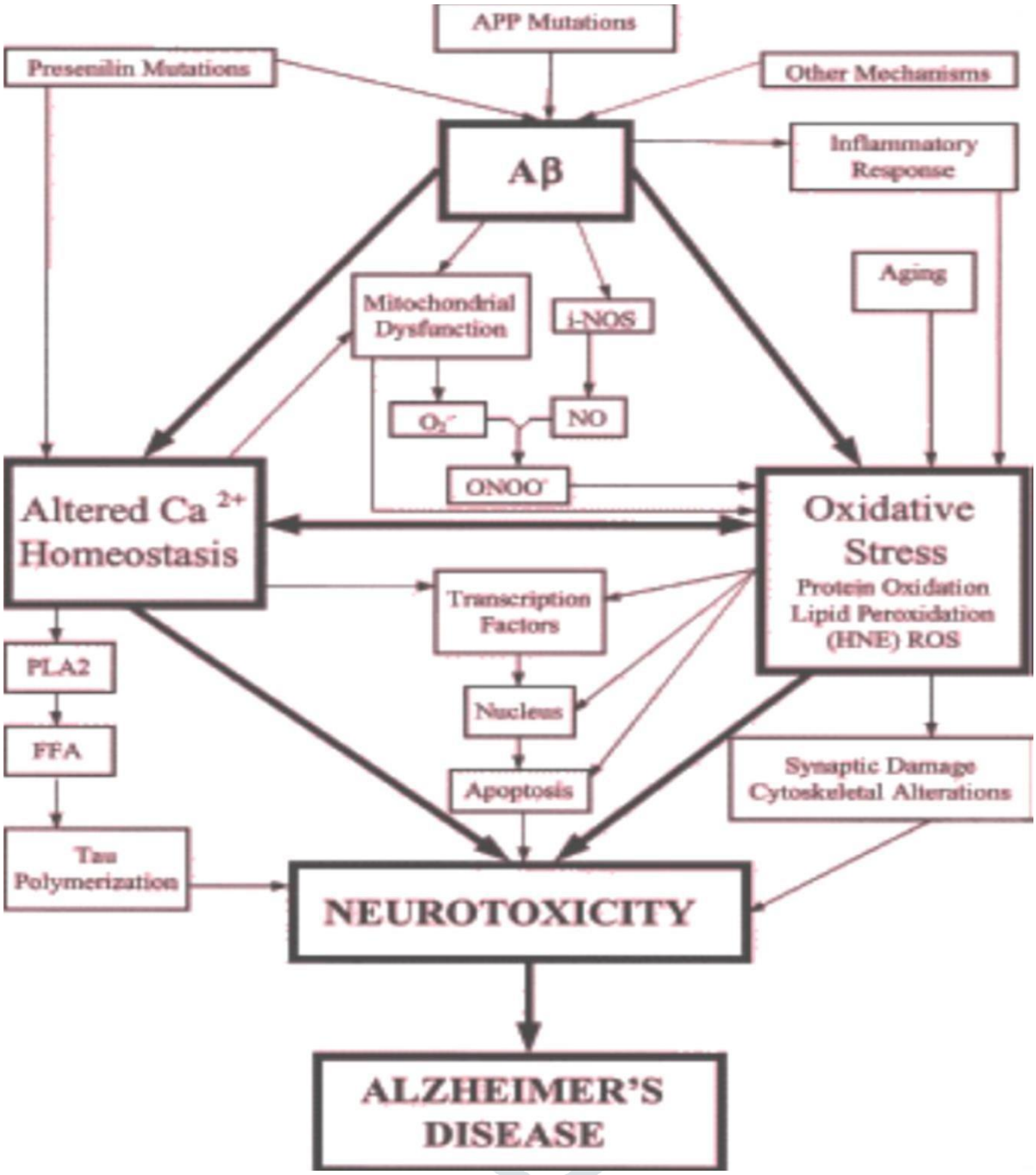


Fig. 1.4: Pathophysiology of AD.

3.3.1.Amyloid Cascade Hypothesis:

Extracellular lesions in the brain and cerebral vasculature are known as neuritic plaques (also known as amyloid or senile plaques). Plaques from AD brains are mostly made up of a protein known as AP. The amyloid hypothesis proposed in 1991 that beta- amyloid (A) deposits are the primary cause of Alzheimer's disease30. In addition, APOE4, a particular isoform of apolipoprotein, is a substantial genetic risk factor for Alzheimer's disease. Apolipoproteins increase the breakdown of beta-amyloid, resulting in an overabundance of amyloid in the brain31. This notion was modified in 2009, with the suggestion that a near relation of the beta-amyloid protein, rather than the beta-amyloid protein itself, might be a main cause of the disease32. According to the

amyloid cascade theory, disrupted APP processing caused AP synthesis, AP caused plaques, plaques caused neurodegeneration, and this neuronal loss caused the clinical dementia symptoms associated with AD[6].

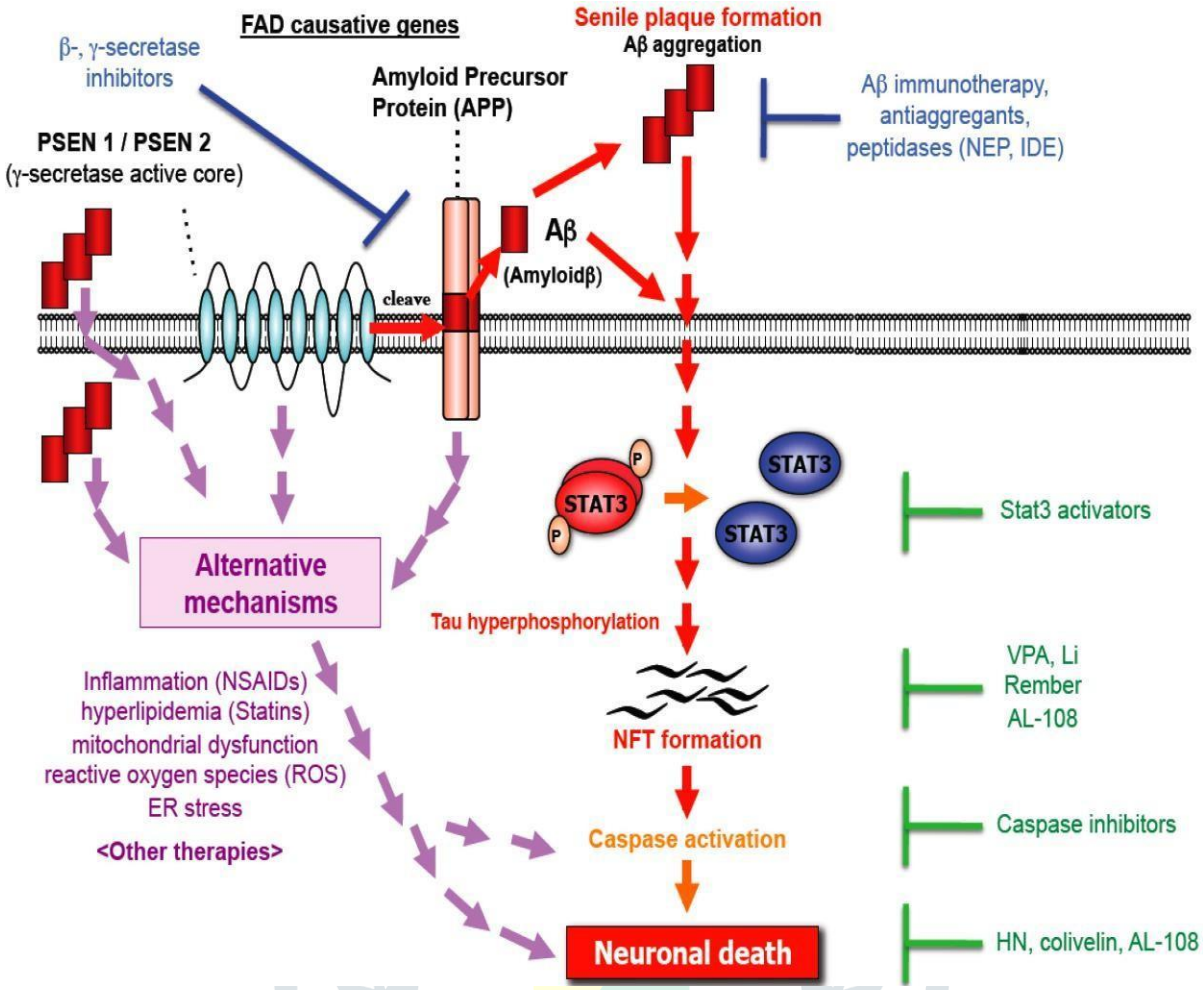


Fig. 1.5: Mechanism involved in neuronal death

3.3.2.Neurofibrillary Tangles:

While -AP was being discovered in plaques, other researchers discovered that NFTs, which are composed of abnormally hyperphosphorylated tau protein, are widely seen in the cells of the hippocampus and cerebral cortex in people with Alzheimer's disease. Microtubules, the cell's transportation, and skeletal support system are supported structurally by tau protein26. Within dystrophic neurons, tangles are intracellular deposits of the microtubule-associated protein tau (t). Tau is generally abundant in neurons, where it binds tubulin monomers to create stable polymers that are thought to be important for cellular transport and axonal development. Tau gets hyperphosphorylated in Alzheimer's disease tangles, resulting in less effective microtubule binding. Unbound tau then clumps spontaneously form insoluble paired- helical filaments, which appear as deposits in neurons33. The cell cannot operate correctly without the entire system of microtubules and finally dies. Because NFTs are a marker of neuronal death, their density corresponds strongly with the degree of dementia[6].

3.3.3.Cholinergic Hypothesis:

In Alzheimer's disease, several neural pathways are disrupted. Any nerve cell population found in or moving through plague-infested regions is damaged. Several neurotransmitter impairments emerge from widespread cell desolation, with cholinergic abnormalities being the most apparent. Cholinergic activity loss is linked to the severity of Alzheimer's disease. In late Alzheimer's disease, the number of cholinergic neurons decreases, and nicotinic receptors in the hippocampus and cortex are lost. Presynaptic nicotinic receptors regulate the release of acetylcholine, as well as glutamate, serotonin, and norepinephrine, all of which are critical for memory and mood. Following the revelation of widespread cholinergic cell death, a cholinergic hypothesis linking AD pathogenesis was developed. The cholinergic hypothesis proposed that memory and cognitive impairment in Alzheimer's disease are caused by the loss of cholinergic cells. As a result, it was anticipated that improving cholinergic function would alleviate memory loss symptoms[6].

3.3.4.Inflammatory Mediators:

The amyloid cascade theory is frequently seen as a consequence of inflammatory or immunologic paradigms. Certainly, amyloid accumulation in the brain is linked to local inflammatory and immunologic changes. Although AP may cause direct neurotoxicity, the inflammatory/ immunologic theories propose that at least some of its toxicity is caused by a -AP protofibril-induced microglia activation and astrocyte migration. This inflammatory reaction might be an attempt to remove the amyloid buildup. It is, however, linked to the production of cytokines, nitric oxide, and other reactive species, as well as complement factors, all of which can harm neurons while also promoting continuing inflammation. Multiple cytokines and chemokines are shown to be increased in AD brains, and specific pro-inflammatory gene variants have been linked to AD6. The deposition of beta-amyloid peptides into amorphous deposits is the initial and most common pathological alteration in Alzheimer's disease. The deposition causes an inflammatory response, which results in an acute phase response including IL-1 and IL-6 in brain parenchymal microglial cells and TGF-beta1 in perivascular astrocytes. The cytokines cause the surrounding astrocytes to produce a variety of secretory proteins that expedite the transformation of diffuse beta-amyloid in amorphous deposits into mature amyloid filaments. The polymerization of beta-amyloid peptides into filaments is accelerated by astrocyte synthesis of beta-amyloid and pathological chaperones such as ACT and apoE. Extracellular matrix proteins might help to keep amyloid formations stable. Finally, human brain neurons respond to glial activation and mature amyloid deposits by forming tangles, followed by neuritic degeneration, and finally cell death[34].

3.3.5.Genetics of Alzheimer Disease:

The APP gene on chromosome 21, the presenilin-1 (PS1) gene on chromosome 14, and the presenilin-2 (PS2) gene on chromosome 1 are known to be significant in the etiology of the early-onset familial disease. On chromosome 19, apolipoprotein E is a significant risk factor for sporadic Alzheimer's disease. A. Mutations in the APP - amyloid is the major protein component of the extracellular plaque. Soluble -amyloid is a natural

component of the human brain that is produced by two enzymes named - secretase and secretase cleaving the bigger APP. A is prevented by an alternate proteolytic route involving -secretase. -Amyloid in the brain is a diverse mixture of

peptides with lengths ranging from 39 to 43 amino acids. - Amyloid with a size of 40 amino acids is known as A40 and is the most common kind. The 42 and 43 amino acid variants are referred to as A42 and A43, respectively, and their quantities grow in the amyloid plaques of Alzheimer's disease brains. Normal APP processing appears to be altered by mutations in the APP that induce increased synthesis of A42 and A43, which are known to cause some forms of autosomal dominant Alzheimer's disease. Patients with Down syndrome, a disorder caused by an extra copy of part or all of chromosome 21, have a different type of APP defect. Down syndrome patients are mentally handicapped and have a variety of developmental problems that are noticeable early in life. Many people acquire dementia in their mid-twenties, with extensive deposition of
-amyloid in plaques and tangles that resemble those observed in Alzheimer's disease33.

B. Mutations in Presenilin Mutations in the presenilin genes are the most prevalent recognized cause of familial Alzheimer's disease. Presenilin proteins are transmembrane proteins found mostly in the endoplasmic reticulum and Golgi apparatus33. Mutations in a gene on chromosome 14 that encodes a protein called presenilin 1 are responsible for the majority and most severe early-onset cases. Presenilin 2, a structurally identical protein, is generated by a gene on chromosome 1. Presenilin 1 and 2 are both genes for membrane proteins that may be involved in the processing of amyloid precursor protein (APP). More than 160 variants in presenilin genes have been discovered, and these mutations appear to limit the activity of - secretase, an enzyme involved in the synthesis of -amyloid peptide (AP)6. Polymorphism of the e4 allele of apolipoprotein E Apo E is a key lipoprotein in the blood that has a role in cholesterol metabolism. The ApoEgene has three naturally occurring alleles, e2, e3, and e4, each of which differs from the others by a single codon33. The Apolipoprotein E (Apo E) genotype is expected to have a major role in sporadic, late-onset AD vulnerability. The apo E gene is found on chromosome 19 and is responsible for its synthesis.[26]

3.3.6.Brain Vascular Disease and High Cholesterol:

All cardiovascular risk factors, including hypertension, elevated low-density lipoprotein cholesterol, decreased high-density lipoprotein cholesterol, and, in particular, diabetes, are also risk factors for dementia. Clogged blood vessels may obstruct nutrient delivery to neurons, and AP removal from the brain may be hindered.

Additionally, vascular disease may expedite amyloid deposition and increase amyloid toxicity in neurons. Controlling high blood pressure is linked to a slower rate of dementia development. Diabetes might increase the risk of dementia due to factors including "metabolic syndrome" (hypertension and dyslipidemia), the influence of potentially harmful glucose metabolites on the brain and vascular system, and insulin. AD has been associated with disruptions in insulin-signaling pathways in both the peripheral and the brain. Insulin may potentially have a role in AP and tau protein6 metabolism.

3.3.7.Free Radical:

Free radicals are produced in the brains of Alzheimer's patients through sources that are specific to the AD brain. The -amyloid peptide and complex glycation end products have been proven to be sources of free radical generation. The -amyloid peptide is the major component of senile plaques, which are common in AD patients' brains. The - amyloid peptide produces free radicals as it leaves the neurons, and these free radicals have been demonstrated to be cytotoxic to hippocampus cells and synaptosomal membranes. There is mounting evidence that free radical-induced oxidative damage may play a role in Alzheimer's disease etiology. Free radicals are oxygen-based reactive molecules that may harm lipids, proteins, and DNA. Because of its high quantity of easily oxidized fatty acids, intensive usage of oxygen, and low quantities of antioxidants, the brain is particularly vulnerable to oxidative damage. Postmortem brain tissue and live individuals with Alzheimer's disease35 have both shown evidence of oxidative damage.

3.4. Various stages of AD:

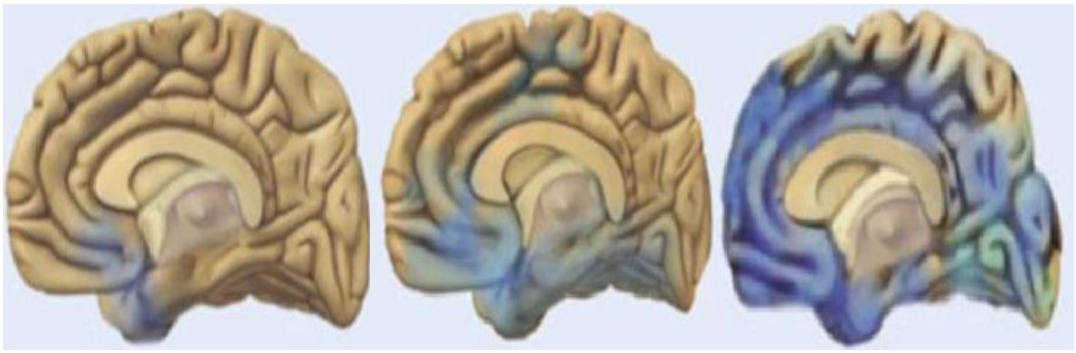
Stage-I (Pre-Dementia): A modest cognitive symptom that manifests itself over two months and is characterized by acts such as attentiveness, planning, abstract reasoning, and semantic remembering.

Stage-II (Early-Dementia): This is a limited cognitive symptom that appears after 20 years before diagnosis and is characterized by learning, memory, fine motor task impairment, language issues, and episodic memory and vocabulary impairments.

Stage-III (Moderate-Dementia): This is an advanced stage of moderate cognitive impairment that lasts 1 to 5 years and is characterized by a gradual deterioration,

increased language issues, deteriorating memory problems, wandering, sundowning, anosognosia, and urine incontinence, among other things.

Stage-IV (Advanced-Dementia): This is a complete stage that lasts more than ten years and involves complete reliance on caregivers. It is the advanced stage of the disease, characterized by bedriddenness, inability to eat, and g.i.t. internal issues (ulcer development and pneumonia)36.



Preclinical Mild to moderate AD Severe AD

Fig. 1.6: Stages of AD

3.5. Risk factors associated with AD:

- The risk of Alzheimer's disease increases with age, doubling every five years beyond the age of 65.
- Other well-known risk factors include dementia in the family and Down syndrome37.
- There's also some indication that persons who have lost consciousness as a result of a head injury are more likely to acquire AD[38].
- Hypertension has also been related to an increased risk of Alzheimer's disease in several studies. As a result of these findings, it was proposed that antihypertensive drugs might reduce the risk of dementia or Alzheimer's disease. Research of almost 5000 men and women over the age of 65 indicated that those who used blood pressure drugs had a considerably decreased chance of developing AD[39].• Vascular disease was establishing itself as the leading cause of senile dementia40.

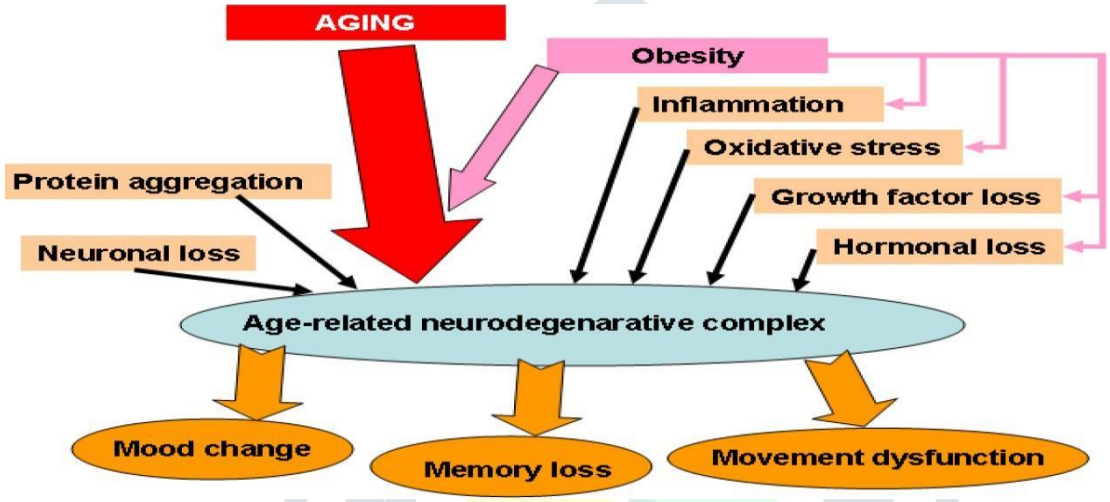


Fig. 1.7: Risk factor associated with AD

3.6. Diagnosis of Alzheimer's disease:

Alzheimer's disease is often diagnosed clinically based on the patient's history, collateral history from relatives, and clinical observations, with the presence of certain neurological and cognitive symptoms and the lack of other illnesses. Advanced medical imaging, such as computed tomography (CT) or magnetic resonance imaging (MRI), as well as single-photon emission computed tomography (SPECT) or positron emission tomography (PET), can assist rule out other forms of dementia or subtypes.[41]

3.7. Treatment of Alzheimer's disease:

The major objective of treatment for Alzheimer's disease is to alleviate symptoms and preserve patient function for as long as feasible. Secondary objectives include addressing the psychological and behavioral consequences of the condition.

3.7.1.Non-pharmacologic treatment:

Because Alzheimer's disease has a significant effect on both the patient and the family, suitable non-pharmacologic and pharmacologic therapy are required. The current primary strategies for the management of Alzheimer's disease are non-medication approaches. When possible, behavioral therapies rather than drugs are used to treat symptoms including sleep difficulties, wandering, urine incontinence, agitation, and aggression in dementia patients.[6]

3.7.2.Pharmacologic therapy

The US Food and Drug Administration (FDA) has approved five prescription drugs to treat the symptoms of Alzheimer's disease, despite the fact that there is no cure. Donepezil, galantamine, rivastigmine, and tacrine are examples of "Cholinesterase Inhibitors." These drugs prevent the breakdown of a chemical messenger in the brain that is essential for learning and memory. The final drug, memantine, regulates the activity of a specific chemical messenger in the brain that is similarly important for learning and memory [4].

Table No. 1: List of cognition-enhancing drugs acting at neurotransmitter level.

SN	Category	Name	Mechanism	Comment
1	Cholinergic agent	Donepezil Galantamine Rivastigmine	Acetylcholinesterase inhibitor Acetylcholinesterase inhibitor, also a possible cholinergic agonist Acetylcholinesterase and butyrylcholinesterase inhibitor	Symptomatic treatment of AD, Vascular dementia, and dementia associated with PD.
2	Glutaminergic agents	D-cycloserine Ampakine Memantine	Partial NMDA agonist enhances glutamate signaling	Significant broad benefits in moderate to severe AD, vascular dementia, and combined nonspecified dementia.

3	Nicotinic agonist	Nicotine	Ach agonist and releaser	Facilitates learning/memory performance

4	Monoamines and agents acting on them	Methylphenidate Modafinil	Effects on catecholamines, serotonin, glutamate, GABA, orexin, and histamine systems	Improve cognition in children and adults with ADHD.
5	Adenosine and phosphodiesterase	Rolipram	Selective type-4 phosphodiesterase inhibitor	Improve LTP.

1.4.8.Oxidative Stress:

Oxidative stress is caused by an imbalance in the prooxidant and antioxidant systems. Oxidative stress can cause reversible or permanent structural, functional, and stability changes in sensitive proteins. Protein modifications such as carbonylation, nitration, and protein-protein cross-linking are frequently connected to a loss of function and can cause damaged proteins to unravel or degrade, or aggregation leading to cytoplasmic inclusions, as seen in age-related neurodegenerative diseases. Proteins that have been oxidized are especially sensitive to the proteasome's proteolytic degradation.

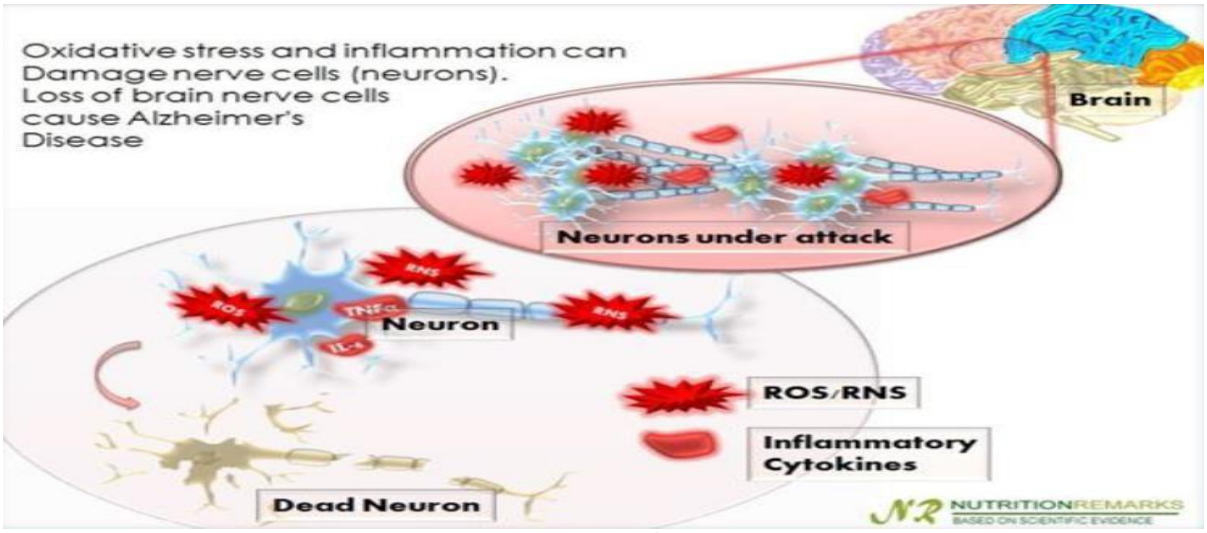


Fig. 1.8: Involvement of oxidative stress in neuronal death

Plant profile 2.1.Taxonomic classification[45]

Kingdom: Plantae

Phylum: Tracheophyta

Class: Equisetopsida C. Agardh Order: Malvales Juss

Family: Bixaceae Genus: Bixa

Species: Bixa Orellana L

2.1. Synonyms[45]

Bixa acuminata Boj, Bixa katangensis Delpierre, Bixa odorata Ruiz & Pav. ex G.Don, Bixa orleana Noronha, Bixa americana Poir, Bixa acuminata Boj, Bixa katangensis Delpierre, Bixa odorata Ruiz & Pav. ex G.Don, Bixa orleana Noronha

2.1.3. Local names [46]

Arabic:Galaga; Bengali:(latkan); Creole: Chiót,Woukou

English:lipstick tree,arnatotree, annatto tree

Gujarati:Siduri Hindi:Latkan

Malay:Jarakbelanda

Telugu:Jaffar Portuguese:Urucum

Kannada:Rangamali

Malayalam:Kurannumannal Marathi:Sendri

Oriya:Lotions Tamil:Sappiravirai, Japhara

Geographical distribution[47]

Bixa Orellana is a native of Brazil that may also be found in other parts of South and Central America. Karnataka, Andhra Pradesh, Tamil Nadu, Odisha, West Bengal, Gujarat, Maharashtra, Madhya Pradesh, and Chhattisgarh are among the Indian states where it may be found.

Fig. 1.9: Bixa Orellana Plant

Fig. 1.10: Bixa Orellana Seeds

Botanic Description

Bixa Orellana is a 2-8 m tall evergreen shrub or small tree with a trunk up to 10 cm in diameter, robust, smooth, fissured, lenticellate bark, inner bark pinkish towards the exterior with orange sap, somewhat bitter; twigs green with minute, rusty, reddish- brown scales, becoming dark brown. [48]

Leaves Petiole terete, thickened at both ends, 2.5-12 cm long; petiole terete, thickened at both ends, 2.5-12 cm long; petiole terete, thickened at both ends, 2.5-12 cm long; petiole terete, thickened at both ends, 2.5-12 cm long; petiole terete, thickened at both ends, 2.5-12 cm long; petiole terete, thickened at both.

Flowers 8-50 flowered, fragrant, 4-6 cm across; pedicel scaly, thickened at apex, bearing 5-6 large glands; in terminal branching panicles, 8-50 flowered, fragrant, 4-6 cm wide; pedicel scaly, thickened at apex, bearing 5-6 large glands; in terminal branching panicles, 8-50 flowered, fragrant, 4-6 cm wide; pedicel scaly, thickened at a Petals 4-7, obovate, 2-3 x 1-2 cm, pinkish, white or purple-tinted; stamens many, 1.6 cm long; anthers violet; pistil 1.6 cm long, consisting of the bristly 1-celled, superior ovary; style thickened upwards, 12-15 mm long; short, 2-lobed stigma.

Fruit a spherical or widely extended ovoid capsule, 2-4 x 2-3.5 cm, flattened, 2 valved, more or less thickly covered with long bristles when mature, green, greenish-brown or red; seeds numerous, obovoid and angular, 4.5 mm long, with brilliant orange-red fleshy coatings.

Seeds Their form ranges from pyramidal to virtually conical, and they measure 0.3–0.5 cm in length and 0.2–0.3 cm in diameter. The quantity of seeds per capsule varies, with each bivalve capsule containing 30 to 60 seeds on average.[49,50]

Phytoconstituents

Bixin is the most abundant component in seeds, while fat and carbohydrate are also found in minor amounts. Isobixin, beta carotene, ellagic acid, salicylic acid, tomentose acid, Crellin, and bixin are some of the compounds found in isobixin. Many chemical elements have been found and isolated from this plant's seeds, seed coats, and leaves, including carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes, sesquiterpenes, triterpenoids, and other miscellaneous substances.

Traditional remedies[51]

Urucum seeds have been used as a laxative, cardiotonic, hypotensive, expectorant, and antibacterial, among other things. It also contains anti-inflammatory properties for bruising and wounds and has been used to treat bronchitis and promote wound healing. This plant may also be used to make oil. Bronchitis, sore throat, and eye irritation have all been treated with an infusion of the leaves. Soft drinks and febrifuge are made from pulp, which includes the seed. The bark of the root is used to treat fever, while the infusion of the leaves is used to treat jaundice.

2. LITERATURE REVIEW

- The efficacy of methanol leaf and seed extracts to suppress bacterial and fungal strains was investigated. Leaf extracts (MIC=1000 mg/ml) were more effective and had antimicrobial activity against a wide variety of bacteria and fungi, with the highest activity against Salmonella typhi (MIC =31.25 mg/mL) and Acinetobacter species (MIC =31.25 mg/mL) against 10 LG/disc Streptomycin (9 0.3 mm and 20 0.2 mm) used as control, and Trichophyton mentagrophy [52]
- When compared to the conventional bacteriocin drug niacin, crude ethanol leaf extracts demonstrated stronger antibacterial activity against P. aeruginosa (MIC 512 mg/mL) and B. cereus (MIC 4096 mg/mL), while seed extract had MIC values of 128 and 1024 mg/mL, respectively. [53] Braga et al. compared the activity of fruit extract (MIC = 78.0 LG/mL) to standard Amphotericin-B (MIC
- = 0.078 LG/mL) against Cryptococcus neoformans.[54]
- Ethanolic leaf and seed extracts of B. Orellana showed broad-spectrum antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans; the zone of inhibition (mm) was 21.50, 20.00, 19.50, 17.00, 19 The results support the traditional use of B. Orellana in medicine, particularly as a gargle for sore throats and oral hygiene.[55]
- In vitro antibacterial activity against Bacillus pumilus was demonstrated by ethanolic leaf extracts, which were followed by extracts from root and hypocotyls, with zones of inhibition of 21.60, 15.80, and 15.20 for 24 mg/mL concentrations, respectively.[56]

- In 2012, disc diffusion assays were used to test the antibacterial activity of ethanolic, methanolic, acetone, and dimethyl sulphoxide extracts against E. coli,
- K. pneumonia, P. aeruginosa, B. subtilis, B. cereus, and S. aureus. The antibacterial effects were stronger in acetone and DMSO extracts than in ethanol and methanol extracts, with zones of inhibition of 10–14 mm and >14 mm, respectively, against a standard of 25 mg tetracycline. [57]
- The crude extract of B. Orellana hairy roots was tested for anti-plasmodium activity against malaria strains 3D7 and K1, and it showed antimalarial activity in the 15–20 lM range, with no cytotoxicity at the detected doses in the mammalian cell lines used in this study (EC50> 26 lM). [58]
- Ethanolic leaves extract (5 mg/mL) of B. Orellana was tested for antifungal and antibacterial activity in vitro using agar diffusion and tube dilution methods, with zones of inhibition of 15–17 mm for all standard Gram-positive bacteria, including Bacillus subtilis, Staphylococcus aureus, and Streptococcus faecalis while showing only minor activity against Escherichia coli, Serratia market [59]
- Conrad et al. investigated the ability of B. Orellana ethanolic leaf extract to scavenge free radicals. 60 The findings reveal that phytochemicals present in the leaves of B. Orellana protect albino rats from carbon tetrachloride-induced blood and liver toxicity. Several organic and aqueous seed extracts were examined in vitro for their capacity to scavenge reactive oxygen and nitrogen species, and the results were compared to bixin standards. When compared to bixin standards with IC50 values of 3.0, 0.3, 1.0, 3.0, and 3.0 mg/mL, the results showed that ethyl acetate extract, which is primarily composed of hypocretin and caffeoylacid derivative (PC), had significant antioxidant effects with IC50 values of 11.0, 1.0, 3.0, 7.0, and 3.0 mg/mL against H2O2, HOCl, O2, NO
- Utilizing ascorbic acid (vitamin C) as a reference standard, the antioxidant activity of B. Orellana seed extract was assessed in vitro using 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity and iron(III) oxide reducing power. Between 0.25 and 2.5 mg/ml, the percentage decrease varied from 5.5 percent to 48.9% when compared to ascorbic acid (2.9–41.5 percent). Similarly, the reducing power of iron(III) oxide has an excellent linear concentration- dependent relation (R2 =0.9986), which is close to that of ascorbic acid (R2 = 0.9934). The abundance of tannins and flavonoids detected in the early investigation, according to the scientists, might explain the action. 61
- Bixin (2.5 or 5.0 mg/kg BW; 48, 24 h, and 10 min) pretreatment decreased the overall number of chromosomal abnormalities by nearly 33%, suppressed lipid peroxidation, and prevented renal glutathione depletion caused by cisplatin (5 mg/kg BW). [62]
- The inhibitory impact of B. Orellana aqueous extract (150 mg/kg) on histamine- induced paw edema in rat models demonstrated a dose-dependent reduction in histamine-induced paw edema. 63 Furthermore, administration with 50 mg/kg and 150 mg/kg aqueous leaf extract reduced carrageenan, histamine, serotonin, and bradykinin-induced acute and chronic rat paw edema considerably. 64 Bradykinin-induced inflammation is inhibited after treatment with lyophilized leaf extract at doses of 50 mg/kg and 150 mg/kg. There was also a drop in nitric oxide production and vascular endothelial growth factor (VEGF), suggesting that the anti-inflammatory

impact might be linked to the reduction in reactive oxygen species. [65]

- The antiulcer properties of hydroalcoholic leaf extract were investigated in rats with 96 percent ethanol-induced liver injury. At dosages of 200 mg/kg and 400 mg/kg, the leaf extract provided partial gastroprotection. [66]

- The inhibitory impact of B.orellana leaves aqueous extract on histamine- induced paw inflammation in mice demonstrated a considerable reduction in paw volumes and almost normalized peritoneal vascular permeability, which was thought to be supported by the inhibition of other permeability-regulating chemicals (NO and VEGF). [63]
- Bixin (50 mg/mL) from B.orellana was tested for COX-1 and COX-2 enzyme inhibitory activity and showed 19 and 33.60 percent inhibition, respectively, when compared to positive controls ibuprofen (2.52 mg/mL), aspirin(180 mg/mL), Vioxx (1.67 mg/mL), and Celebrex (1.67 mg/mL), which showed 51, 78, 67
- B. Orellana extracts and compounds also have anticancer and antitumor properties. Bixin was regarded to be the most effective chemical. 67 Bixin's cell proliferation inhibitory effects differed amongst tumor cell lines, with IC50 values of 33, 49, 45, and 39 mg/mL for colon, CNS, stomach, and lung cancer cell lines, respectively. Bixin from B. Orellana seeds was tested for a clastogenic and anticlastogenic activity to determine the chromosomal damage caused by clastogen cisplatin. [68]
- In Swiss albino rats, methanol leaf extract (500 mg/kg, crude herb medicinal equivalent, 3 times/day) reduced carbon tetrachloride liver damage. Histopathological analysis of liver tissues revealed a reduction in the elevations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST), and cholesterol by 52 percent, 57 percent, and 53 percent, respectively. 69

- By using silica gel chromatography, a bioactive sesquiterpene (dishware) was extracted from a dichloromethane extract of air-dried leaves of B. Orellana and identified using 1H and 13CNMR. Ishwaranewas were examined for gastrointestinal mobility at doses of 25, 50, and 100 mg/kg. The antitoxic property was detected in the prophylacticassay at 100 mg/kg. Ishwarane caused higher propulsive movement of the gastrointestinal tract (88.38 13.59 percent) than the negative control (78.47 10.61 percent) at a dosage of 50 mg/kg. 70
- A methanol leaf extract (125, 250, and 500 mg/kg, 30 min) was observed to significantly in mice, delaying the intestinal passage of charcoal meal (P 0.01), with 79.55 percent inhibition at a dosage of 500 mg/kg. 71
- The effect of a methanol extract of B. Orellana leaves on diuretics was studied in Wister rat models, and the results revealed that the extract had a diuretic effect, with a significant increase in urine volume (2.4 0.02 mL) and levels of sodium (82 3.07 mEq/L), potassium (12.30.47 mEq/L), and chloride (712.52 mEq/L) when compared to the control group 0.7 0.04 ml [73]
- In mice, ethanol extracts (LD50= 44 LG) provide partial protection against both ropsatrox venom edema forming activity and death. [74]
- This plant's root and leaf extracts were discovered to have substantial antigonorrheal action, with inhibition zones of 6.0 mm and 17.40 mm, respectively. [75]

3. AIM AND OBJECTIVES

The goal of this study is to assess Bixa Orellana's neuroprotective and antioxidant properties in an animal model of scopolamine-induced memory impairment.

The current study's goals were to learn more about the pathogenesis of dementia and the potential utility of these herbs.

1. To carry out extraction and preliminary phytochemical screening of action of *Bixa Orellana* leaves.
2. To carry out pharmacological study of*Bixa Orellana* leaves. in scopolamine-induced dementia.
3. The major goal of this study was to evaluate the neuroprotective and antioxidant effects of Bixa Orellana leaves to the conventional medication donepezil in Wistar albino rats with scopolamine-induced memory impairment.

1. Plan of work

1. Collection and authentication of the plant.
2. Preparation of extract and preliminary phytochemical screening.
3. Acute toxicity study of the plant extract and Drugs and Doses selection.
4. Scopolamine induced memory impairment in animal
5. Treatment of animals with a standardized dose of plant extract.

5. MATERIALS AND METHODS

5.1. Authentication and gathering of plants

Fresh Bixa Orellana leaves were obtained from a local herbal market Ayurvedic raw material shop in Hyderabad's Ranga Reddy district. Botanical Survey of India, Hyderabad.

5.2. Successive extraction [76]

Following the collecting and identification of the selected plant leaves, they were properly washed with running tap water and allowed to drip completely. The cleaned leaves were left to air dry in the shade at room temperature. They were ground into powder once they were completely dry. In a soxhlet extractor, about 30 g of the air- dried powdered drug was extracted sequentially with solvents such as petroleum ether, chloroform, ethyl acetate, and ethanol: The powder was macerated with the above respective solvents for 24 hours in the soxhlet apparatus, and then extracted for up to 6 hours with at least 6-8 siphoning. The powdered material was dried in air and then in

an oven below 50 oC each time before extracting with the next solvent. Each extract was concentrated by removing the solvent and drying it in a tarred glass beaker over a water bath. The extracts from each solvent were weighed, and the proportion of air- dried weight to the bark powder was computed. The process was done three times, and the mean value was calculated, as well as the color and consistency of each extract.

5.3. Preliminary phytochemical screening

Different extractives of the plants were tested for the presence of several phytoconstituents77 in a preliminary phytochemical analysis.

5.3.1.Test for Alkaloids: A few milligrams of each extract were dissolved in 5 milliliters of 1.5 percent v/v HCl and filtered separately. Following that, the filtrates were put through a series of tests:

A) Dragondroffs test: The extract was distributed and dried on Whatman filter paper. Ammonia was used to make the test filter paper alkaline, and chloroform was used to remove it. The chloroform extract was put on dragendroff's reagent-

impregnated filter paper. The presence of alkaloids is indicated by the development of an orange-red color.

B) Mayer's reagent: The extract was treated with Mayer's reagent. The production of a cream-colored precipitate indicates the presence of alkaloids.

C) Wagner's Reagent: Wagner's reagent was used to treat the extract. The presence of alkaloids is indicated by the formation of a reddish yellow precipitate.

D) Hager's reagent: Hager's reagent was used to treat the extract. The presence of alkaloids is indicated by the formation of a yellow-colored precipitate.

5.3.2.Test for Glycosides

Test A: 5 mL dilution to 5 mg extract H2SO4 was added, warmed on a water bath, and then filtered. A 5 percent NaOH solution was used to neutralize the aforesaid solution. A water bath was used to heat 0.5mL Fehling's solution A and 0.5mL Fehling's solution B for 2 minutes.

Test B: 5 mL water was added to 5 mg of extract and warmed on a water bath before filtering. A 5 percent NaOH solution was used to neutralize the aforesaid solution. A water bath was used to heat 0.5mL Fehling's solution A and 0.5mL Fehling's solution B for 2 minutes. The color of the red precipitate produced in tests A and B were compared. The presence of glycosides is indicated if test A has more precipitate than test B. If the same precipitate forms in both tests A and B, glycosides are not present.

5.3.3.Test for Naphthoquinones Juglone test

2mL solvent ether and an equivalent volume of oil. ammonia solution was added to the 5mg extract. The presence of naphthoquinones is indicated by a reddish-pink color.

5.3.4.Test for anthraquinones Bontrager's test

5 mL oil. HCl was added to the 5 mg extract. 2mL solvent ether was added to the solution after it had been heated and cooled. When the ether layer was separated and a strong ammonia solution was added, the ammonia layer became pink, indicating the presence of anthraquinones.

Modified Brontager's test

5 mL oil. HCl and 2-3 drops of FeCl3 were added to the 5 mg extract. 2mL solvent ether was added to the solution after it had been heated and cooled. When the ether layer was separated and a strong ammonia solution was added, the ammonia layer became pink, indicating the presence of C-type anthraquinones glycosides.

5.3.5.Test for Iridoidal glycosides Trim –Hill test

For 3-6 hours, the extract was treated with 5 mL of 1 percent v/v HCl. 0.1 mL of the solution was decanted and transferred to a test tube containing 1 mL Trim-Hill reagent (10 mL acetic acid, 1 mL 0.2 percent copper sulfate, and 0.5 mL conc. HCl), which was heated over a flame, resulting in the creation of blue color, which shows the presence of iridoids.

5.3.6.Test for Coumarin glycosides With ammonia

The presence of coumarins was determined by adding a few drops of extract solution to paper that had previously been treated with ammonia. The development of fluorescence shows the presence of coumarins.

With alkali solution

The presence of coumarins was determined by dissolving the extract in alcohol and alkalinizing it with KOH solution. The development of blue or green fluorescence shows the presence of coumarins.

5.3.7.Test for Cyanogenetic glycosides Grignard's test

The extract was transferred to a flask using sodium picrate paper strips as a stopper. It was made sure that no paper touched the test tube's inner surface. For 30 minutes, the material was warmed up. The presence of cyanogenetic glycosides is shown by the sodium picrate paper becoming red.

5.3.8.Test for Sterols Salkowaski test:

A few milligrams of extract were dissolved in 2 milliliters of chloroform, then 2 milliliters of concentrated H2SO4 were added from the test tube's side. For a few minutes, the contents of the test tube were shaken. The presence of sterols is shown by the development of red color in the chloroform layer.

Liebermann test:

In a test tube, a few milligrams of the extract were combined with acetic anhydride and slowly heated. A few

drops of concentrated H2SO4 were added. The presence of sterols is indicated by the development of a blue color.

5.3.9.Test for Triterpenoids Libermann-Burchard test

A few mg of extract was dissolved in chloroform, followed by 0.5 mL acetic anhydride and conc.H2SO4 from the test tube's side. The presence of triterpenoids is indicated by the creation of a reddish-brown ring at the confluence of two liquids.

5.3.10. Test for Flavanoids Shinoda test

A tiny amount of the extract was diluted in 5 mL of 95 percent ethanol (v/v) and treated with a few drops of conc. HCl and 0.5 g of magnesium turnings. The presence of flavonoids is indicated by the development of pink, magenta, and red colors.

5.3.11. Test for Tannins With FeCl3

A tiny amount of the extract was dissolved in 5mL of 95 percent v/v ethanol and then treated with FeCl3 solution. The presence of hydrolyzable and condensed tannins is indicated by the development of blue and brownish-green colors.

Matchstick Test

Matchsticks were dipped in a methanolic extract solution, dried, and then dipped in conc. HCl. Near the flame, a matchstick was dried. The presence of catechins is indicated by the magenta/pink coloration of the matchstick.

5.3.12. Tests for Carotenoids Carr-price reaction

Antimony trichloride solution was used to treat the extract, and the formation of blue color shows the presence of carotenoids.

5.3.13. Test for carbohydrates Molisch's test:

1mL Molisch's reagent was added to a few milligrams of extract and mixed thoroughly. The presence of carbohydrates is shown by the development of a violet ring at the intersection of two liquids after 2mL of Conc, H2SO4 was introduced from the side of the test tube.

5.3.14. Test for Proteins Biuret test:

In 2 mL of alcohol, 5 mg of extract was dissolved. 2 mL Biuret reagent was added to the alcoholic solution. The presence of protein is indicated by the development of a violet color.

5.4. Screening for amnesic activity

Using healthy Albino rats and Swiss mice (20-25 g) of either sex, several parameters for memory impairment were examined in vivo.

(1) Acute toxicity tests for the fatal and effective doses were conducted.

(2) Learning and memory test:

(a) Elevated as well as a maze test

(3) Memory impairment caused by scopolamine in rats.

(5) Locomotor activity that occurs on its own.

5.5 Drugs/Chemicals

Sigma-Aldrich provided scopolamine, streptozotocin, sodium hydroxide, Triton X-100, acetylthiocholine iodide, sodium chloride (NaCl), bovine serum albumin (BSA), 5,5′- dithiobis(2-nitro-benzoic acid)(DTNB), Folin-hydrochloric Ciocalteu's acid,

trichloroacetic acid (St. Louis, MO, USA). UCB Ltd, Mumbai, India, provided piracetam (Nootropil®).

6. Animals:

Adult male Wistar rats were used in the trials (225-250 g, 8-9 weeks old). Animals were procured from the Central Drug Research Institute's Laboratory Animal Services Division in Lucknow, India. They were housed in polyacrylic cages (22.5-37.5 cm) and kept in regular housing conditions (room temperature of 24-27°C and humidity of 60– 65%) with a 12-hour light/12-hour dark cycle. Food and drink were freely accessible. The study was authorized by the Institutional Animal Ethics Committee (IAEC) following the Committee for Control and Supervision of Experiments on Animals (1088/07/ CPCSEA) regulations, and all experimental procedures were conducted in strict accordance with ethical standards.

7. Determination of acute toxicity studies:

Wistar rats (225-250 g, of either sex (20-30 gm) were used to test the acute toxicity of CM seed oil and EEBA under conventional husbandry circumstances. For toxicity experiments, the animals have fasted for 3 hours before the experiment and the Up and Down Procedure (OECD recommendations number 425) was used. A single dosage of the extract was given to the animals, and they died within 48 hours. The dose for the following animal was calculated using the short-term toxicity profile of the extract, according to OECD standards number 425. 1/20th, 1/10th, and 1/5th dosages were deemed low, medium, and high doses, respectively, for LD50. With a dosage of 2000 mg/kg, there was no mortality.

8. Methods

In the next sections, the experimental protocol for behavioral and memory testing, medication administration, and biochemical research in brain regions is addressed.

8.1. Behavioural Tests

Morris water maze, Social recognition, Pole climbing, and Elevated plus-maze tests were used to assess memory function. The Medicraft Photo Actometer was used to measure locomotor activity in mice and rats.

8.2. Spontaneous locomotor activity

Using a Medicraft Photo Actometer animal activity monitor, the locomotor activity of each rat and group of mice was assessed. Every minute, the activity was counted, and the totals were added for a total of 15 minutes. After the behavioral experiments were completed, the locomotor activity of rats and mice was examined to see if scopolamine, streptozotocin, or other medication treatments influenced animal behavior78-79.

9. Test for learning and memory

Morris water maze test, social recognition test, Pole climbing test, and Elevated plus maze test were used to measure learning and memory. These tests are most typically employed in rats to evaluate their learning and memory skills.

Elevated Plus Maze

The raised plus maze was used to test learning and memory in mice using an exteroceptive behavioral paradigm (where the stimulus was located outside of the body). Two open arms (16 cm x 5 cm) and two covered arms made up the equipment (16 cm x 5 cm x 12 cm).

The arms were stretched from a central platform (5 cm x 5 cm), and the maze was raised to a height of 25 cm from the ground. Each animal was put at the end of an open arm, facing away from the center platform, on the first day. The time it took the animal to move onto one of the covered arms with all four legs was measured as transfer latency (TL). On the first day, TL was recorded. If the animal did not enter one of the covered arms within 90 seconds, it was gently forced into one of the two covered arms, with the TL set to 90 seconds. The animal was given 10 seconds to explore the maze before being returned to its home cage. On the second day80, memory retention was tested 24 hours following the first-day trial.

10. Experimental models of memory impairment

In rats, memory impairment was generated by intraperitoneal administration of scopolamine81, while in mice, memory impairment was induced by intracerebral injection of streptozotocin[82].

a) Scopolamine-induced memory impairment in mice.

A muscarinic receptor antagonist, scopolamine (3 mg/kg), was diluted in normal saline (0.9 percent NaCl) and given intraperitoneally in a volume of 1 ml/kg body weight. Behavioral testing was performed on rats 5 minutes after scopolamine injection[82].

11. Animal Grouping

Rats were used in the experiments. The scopolamine-induced amnesia paradigm was performed on rats, whereas the STZ-induced memory deficit experiment was performed on mice.

A) Rats are grouped.

B) The rats were split into groups of five at random.

Group 1 : (1 ml/kg, i.p.) vehicle treatment

Group 2: Treatment with scopolamine (3 mg/kg, i.p.)

Group 3: Piracetam (100 mg/kg, i.p.) with scopolamine (three milligrams per kilogram, i.p.)

Group 4 : EEBO (100 mg/kg, p.o.) + Scopolamine (3 mg/kg, i.p.) Group 5 : EEBO (200 mg/kg, p.o.) + Scopolamine (3 mg/kg, i.p.)

12. Drug administration

A) Drug administration in Scopolamine model

Piracetam, a commonly used nootropic, was utilized to verify the scopolamine-induced memory impairment paradigm. It was given i.p. at a dosage of 100 mg/kg for one week. The EEBA and CM seed oils were evenly suspended in a solution of 1 percent carboxymethyl cellulose (CMC) in water and given orally (p.o.). For one week, they were given dosages of 100 and 200 mg/kg. The vehicle group received CMC (10 mL/kg, p.o.) for 1 week and then normal saline (i.p.) on the last days. To elicit memory impairment in rats, scopolamine was administered 1 hour after the administration of vehicle, piracetam, or extracts.

13. Biochemical Estimation

Following the conclusion of behavioral investigations, the biochemical parameters of oxidative stress and cholinergic function were calculated in all drug treatment groups. As stated in the next section, these calculations were made in the cerebellum, cortex, and hippocampus.

1. Brain collection and dissection into cortex and hippocampus

Tota et al., 2009, 201278, 82 explained how the cortex, cerebellum, and hippocampus were separated from the brain.

2. Brain tissue Preparation:

Animals were slaughtered with an overdose of ether anesthesia for biochemical tests. The brain was quickly removed, placed on an ice-cold plate, and then dissected into the hippocampus, cortex, and cerebellum using a method based on Glowinski and Iversen et al., 196619. For GSH, MDA, and acacetylcholinesterase, the brain was homogenised in sodium phosphate buffer (0.03 M, pH-7.4, 10% w/v) using an IKA homogenizer at a speed of 9500 rpm.

A) Acetylcholinesterase activity in Cortex, Cerebellum, and Hippocampus

The serine protease acetylcholinesterase (AChE) hydrolyzes the synapse acetylcholine. Hurt is for the most part situated in neuromuscular intersections and cholinergic mind neurotransmitters, where its activity assists with halting synaptic transmission. It is a member of the carboxylesterase enzyme family.

Principle of Assay

The acetylcholinesterase assay is based on the Ellman technique, in which thiocholine generated by acetylcholinesterase reacts with 5,5'-dithiophosphate to give a yellow color (2-nitrobenzoic acid). The enzyme activity in the sample is proportional to the intensity of the product color, which is measured at 412 nm.

Procedure:

The brain homogenate was combined with 1 percent Triton X-100 and centrifuged at 10,000 g for 60 minutes at 4°C. The supernatant was collected and utilized to determine the amount of acetylcholinesterase (AChE). 0.4 mL supernatant, 2.4 mL phosphate buffer (pH 8), 20 l acetylthiocholine iodide, and 100 l DTNB were used in the test. Using a spectrophotometer, the change in absorbance was recorded for 10 minutes at 2-minute intervals at 412 nm. The activity of AChE was measured in millimoles per minute per milligram of protein78.

The following formula was used to compute AChE's specific activity: E x 1000 x V / 1.36 x 10000 x v AChE activity

ΔE = Extinction change / min 1000 is the mole conversion factor.

V is the total volume of the reaction mixture.

Extinction = 1.36 x 10000 v = volume of enzyme (supernatant) used as a coefficient The number of micromoles of acetylthiocholine iodide hydrolyzed per minute per milligram of protein was used to define one unit of acetylcholinesterase activity. Acetylcholinesterase-specific activity is measured in moles/min/mg protein.

B) MDA Estimation in cortex, cerebellum, and hippocampus

The chemical molecule malondialdehyde (MDA) has the formula CH2(CHO)2. This reactive species is a sign of oxidative stress that occurs spontaneously. MDA is mostly found as an enol:

HOCH=CH-CHO=CH2(CHO)2

MDA is a highly reactive chemical that is seldom seen in its pure state. The mechanism is based on the oxidation of unsaturated lipids, which produces more radical species as well as hazardous by-products that might affect the host system. In an oxidizing environment, polyunsaturated lipids are particularly vulnerable to this sort of damage, and they can react to generate lipid peroxides. Lipid peroxides are unstable in and of themselves, and they decompose further to produce a complicated succession of chemicals, including reactive carbonyl compounds. MDA is formed when polyunsaturated fatty acid peroxides react further.

Principle of Assay

The interaction of (MDA) with thiobarbituric acid (TBA), results in the formation of the MDA-TBA2 adduct, which absorbs significantly at 532 nm.

Procedure:

MDA, a spectrophotometric measurement of lipid peroxidation, was used. Following homogenization, the tissue homogenate was combined with 30 percent TCA, 5N HCl, and then 2 percent TBA in 0.5 N NaOH. The mixture was heated to 90°C for 5 minutes before being centrifuged for 10 minutes at 6000 pm. Using a UV

spectrophotometer80, the pink color of the supernatant was measured at 532 nm.

C) GSH estimation in Cortex, Cerebellum, and Hippocampus.

Glutathione (GSH) is a tripeptide containing a gamma peptide bond between the cysteine amine group and the glutamate side-chain carboxyl group. It acts as an antioxidant, preventing reactive oxygen species such as free radicals and peroxides

from causing harm to critical cellular components. In animal cells, thiol groups are reducing agents that occur at a concentration of around 5 mm.

By acting as an electron donor, glutathione converts disulfide bonds produced inside cytoplasmic proteins to cysteines. Glutathione is transformed into glutathione disulfide, which is an oxidized form. Glutathione may be reduced back to its original state by glutathione reductase, which uses NADPH as an electron donor. The ratio of reduced glutathione to oxidized glutathione in cells is frequently utilized as a toxicity indicator[84].

Principle of Assay

5-5'-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman's Reagent) interacts with GSH to produce 5-thionitrobenzoic acid (TNB) and GS-TNB, a 412 nm chromophore. Glutathione reductase and -nicotinamide adenine dinucleotide phosphate (NADPH) decrease the GS-TNB, releasing a second TMB molecule and recycling the GSH, enhancing the reaction. Any oxidized GSH (GSSG) in the reaction mixture or produced by the mixed disulfide reaction of GSH with GS-TNB is quickly converted to GSH. **Procedure:**

The reaction of GSH with 5-5'-dithiobis 2-nitrobenzoic acid (DTNB) at 412 nm was used to measure its antioxidant level. The processed tissue sample was treated with a 5 percent TCA solution in an equivalent amount. After centrifuging for 10 minutes at

3.000 rpm, 0.05 ml of supernatant, 0.1 ml phosphate buffer (pH-8.4), DTNB, and 0.05 ml distilled water were added to the mixture. The absorbance was then measured spectrophotometrically at 412 nm in a UV spectrophotometer78 within 15 minutes.

14. Statistical Analysis

Student t-test (for scopolamine/STZ vs. control and piracetam vs. scopolamine/STZ) and one-way Analysis of Variance (ANOVA) followed by Turkey test (for treatment groups vs. scopolamine/STZ) were used in the statistical analysis of behavioral and biochemical values.

6. RESULTS

6.1. Phytochemical investigation

Table No.6.1: Phytochemical screening of leaves of *Bixa Orellana*

Phytochemicals	Leaves extract of Bixa Orellana			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids	+	+	-	+
Flavonoids	-	+	+	-
Steroids	+	-	-	+
Cardiac glycosides	-	-	+	+
Saponins	-	+	-	+
Terpenoids	-	+	-	-
Amino acids	+	-	-	+
Carbohydrates	-	-	-	+
Proteins	+	+	-	+
Phobatinins	-	-	-	-

+ Present; – Absent

6.2 Acute toxicity and dose determination

An acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino mice were kept for overnight fasting before drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, the bodyweight of methanol extracts of ***Bixa Orellana*** *leaves*. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions, etc. Mortality, if any, was determined over 2 weeks. Hence the selected dose was 100mg/kg and 200mg/kg of body weight.

3. Effect of *Bixa Orellana* on locomotor activity

There was no discernible difference in spontaneous locomotor activity across the groups.

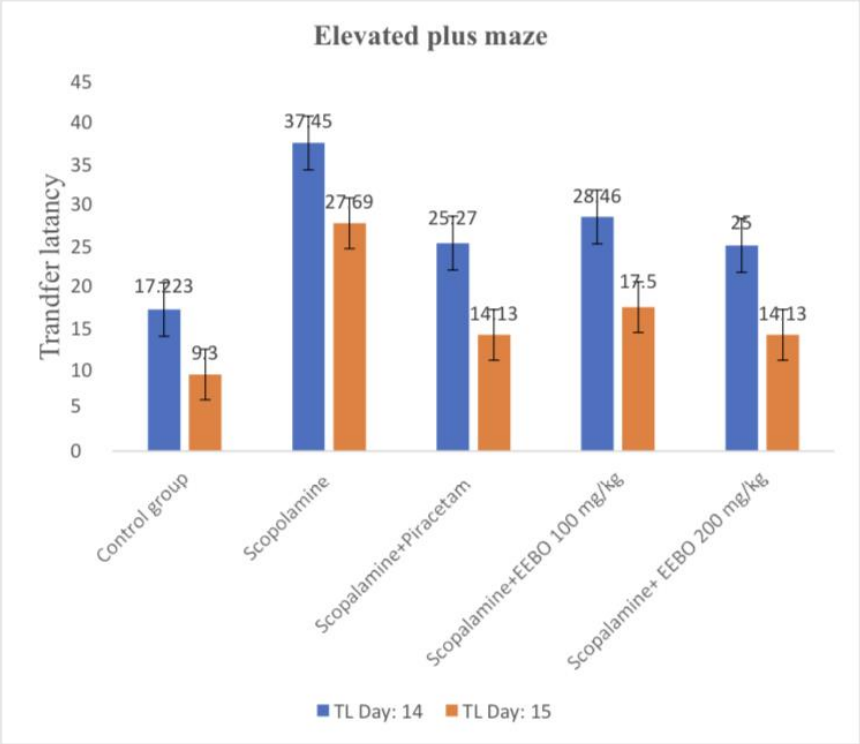
4. Effect of *Bixa Orellana* scopolamine-induced memory impairment in Elevated plus maze test

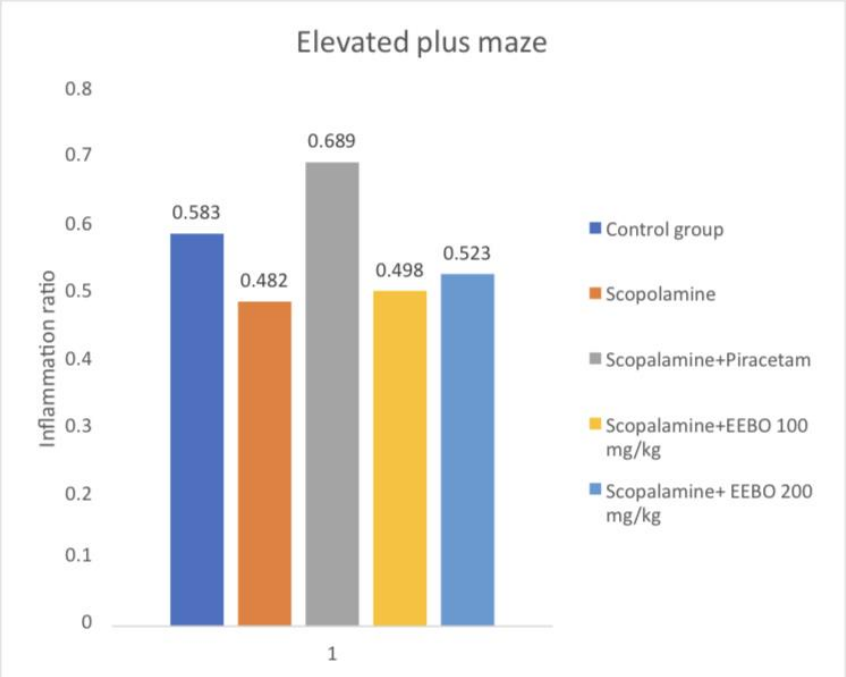
At the end of the 14th day, the impact of all drug-treated groups was assessed. TL was captured on tape. On the 15th day, TL for all of the drug-treated groups was significantly lower than on the 14th day. Exaggerated IR implies an improvement in psychological traits and memory impairment, whereas decreased IR suggests the creation of a state of mind. Animals in the negative control group (scopolamine) shrank significantly more than those in the other groups, indicating that a state of mind was induced. Furthermore, when compared to intermediate and low doses independently, the high dosage exhibited a rise in IR, and the extract was the only group that showed a significant increase when compared to the negative group.

Table 6.4: Effect of Effect of ethanolic leaves extract of *Bixa Orellana* IR

Groups	IR	**TL Day: 14**	**TL Day: 15**
Group I (Control group)	0.583 ±0.068	17.223	9.3
Group II (scopolamine(0.3 mg/Kg)	0.482±0.115	37.45	27.69
Group III (scopolamine +Piracetam(100 mg/kg))	0.689±0.075	25.27	14.13
Group IV (scopolamine +EEBO, 100 mg/kg)	0.498±0.046	28.46	17.5
Group V (scopolamine +EEBO, 200 mg/kg)	0.523±0.121	25	14.13

Values are expressed as the mean±standard error of the mean of n=6 rats/treatment. Significance **p≤0.01. TL: Transfer latency, IR: Inflexion ratio


Elevated plus maze
Trandfer latancy
45
40
35
30
25
20
15
10
5
0
17.223
9.3
37.45
27.69
25.27
14.13
28.46
17.5
25
14.13
Control group
Scopolamine
Scopalamine+Piracetam
Scopalamine+EEBO 100 mg/kg
Scopalamine+ EEBO 200 mg/kg
TL Day: 14
TL Day: 15


Elevated plus maze
Inflammation ratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.583
0.482
0.689
0.498
0.523
1
Control group
Scopolamine
Scopalamine+Piracetam
Scopalamine+EEBO 100 mg/kg
Scopalamine+ EEBO 200 mg/kg

5. Biochemical estimation

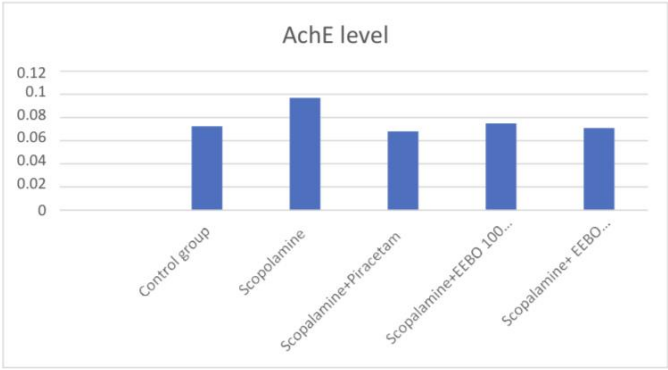
5.1. Estimation of acetylcholinesterase enzyme levels in the brain

As seen in Table 6.5.1, all of the groups had lower acetylcholinesterase accelerator activity as compared to scopolamine. Scopolamine significantly increased the activity of the acetyl enzyme as compared to the control group. The drug-treated groups significantly decreased the increase in AChE activity caused by scopolamine. When compared to low and medium dosage mice, high-dose animals exhibited a significant drop in acyl-enzyme activity, whereas extract-alone animals showed a significant decrease in acetylcholinesterase activity. The reduction in acetylcholinesterase enzyme activity improved cognition and had anti-properties. Alzheimer's

Table 6.5.1: Effect of ethanolic leaves extract of *Bixa Orellana* acetylcholine esterase level

Groups	Acetylcholine esterase level
Group I (Control group)	0.0724±0.001
Group II (scopolamine(0.3 mg/Kg)	0.097±0.001
Group III (scopolamine +Piracetam(100 mg/kg))	0.068±0.00115
Group IV (scopolamine +EEBO, 100 mg/kg)	0.075±0.0025
Group V (scopolamine +EEBO, 200 mg/kg)	0.071±0.0001

Values are expressed as the mean±standard error of the mean of n=6 rats/treatment. Significance **p≤0.01



5.2. Determination of MDA level

When scopolamine was given, the MDA level in the total brain was significantly higher than in the control group. There was a significant (p0.001) reduction in protein levels in the treated groups at 100 mg/kg, 150 mg/kg, and 200 mg/kg. MDA levels were significantly lower in the donepezil-treated group. Table 5.4 shows the results.

Table 6.5.2: Effect of ethanolic leaves extract of *Bixa Orellana* MDA level

Groups	MDA level
Group I (Control group)	0.0193±0.002
Group II (scopolamine(0.3 mg/Kg)	0.034±0.0005
Group III (scopolamine +Piracetam(100 mg/kg))	0.009±0.00025
Group IV (scopolamine +EEBO, 100 mg/kg)	0.017±0.0006
Group V (scopolamine +EEBO, 200 mg/kg)	0.013±0.000

The mean and standard error of the mean of n=6 rats/treatment is used to calculate the values. **p≤0.01

5.3. Determination of glutathione peroxidase

In human cells, glutathione is important for maintaining proper function and reducing aerophilic stress. It was designed to scavenge chemical group radicals, singlet oxygen, and a variety of electrophiles. Glutathione reductases, which catalyze the reduction of GSSG to GSH, keep the equilibrium narrow. The cell's chemical

response is triggered by a drop in glutathione levels. In the negative group, the glutathione level in the brain drops dramatically. Ethanolicleaves extract in dosages treated with Bixa Orellana (100 and 200 mg/kg) exhibited a significant (0.01) rise in glutathione levels. The glutathione level in the standard group of rats increased significantly. In comparison to the control group, the group that did not receive scopolamine showed an increase in glutathione levels.

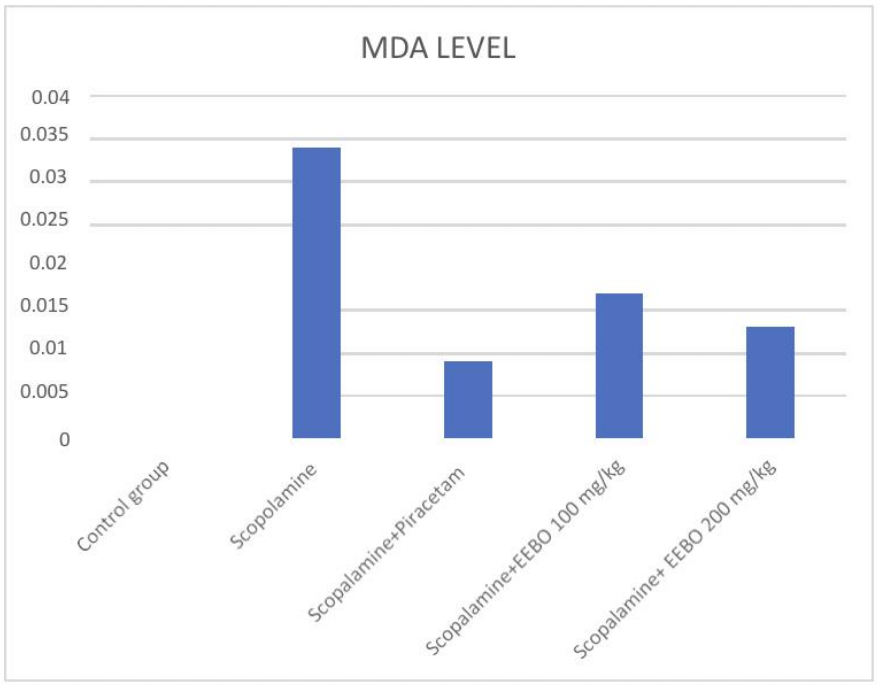
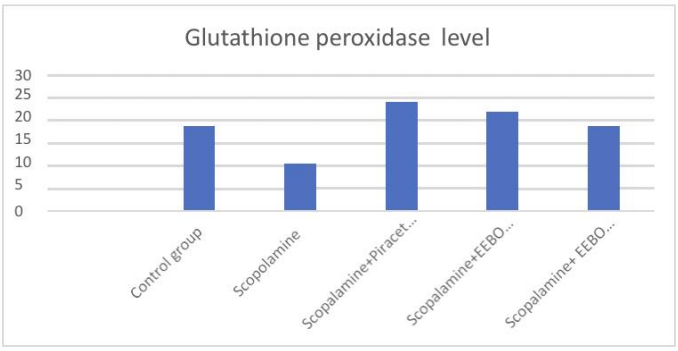


Table 6.5.3: Effect of ethanolic leaves extract of *Bixa Orellana* glutathione peroxidase level

Groups	Glutathione peroxidase level
Group I (Control group)	18.69 ± 4.24
Group II (scopolamine(0.3 mg/Kg)	10.51 ± 3.94
Group III (scopolamine +Piracetam(100 mg/kg))	24.06 ± 1.32
Group IV (scopolamine +EEBO, 100 mg/kg)	21.93 ± 1.75
Group V (scopolamine +EEBO, 200 mg/kg)	18.74 ± 2.16



Values are expressed as the mean±standard error of the mean of n=6 rats/treatment. Significance **p≤0.01

7. DISCUSSION

Dementia is a substantial loss of cognitive capacity in a previously healthy individual that goes beyond what is usual with aging. Dementia is a non-specific condition that affects cognitive functions such as memory, attention, language, and problem-solving. Alzheimer's disease (AD) is the leading cause of dementia in people over the age of 65. This condition most commonly affects those over the age of 65 years85. The illness is defined neuropathologically by the development of many senile plaques and neurofibrillary tangles, which contribute to neuronal degeneration86. Although the etiology of Alzheimer's disease is complicated, growing data suggests that cholinergic deficiency is directly linked to the severity of cognitive impairment and memory loss in AD

patients[87]. Furthermore, oxidative stress is a significant contributor to the pathogenesis of AD[88].

Studies with scopolamine-induced memory impairment in rats:

The central cholinergic system has been shown to have a significant role in the regulation of cognitive function[89]. Cholinergic neurons degenerate rapidly in Alzheimer's disease patients[90]. Increasing endogenous acetylcholine levels with AChE inhibitors is now the most effective treatment for Alzheimer's disease[91]. Learning and memory processes are disrupted in rodents[92], nonhuman primates[93], and humans[94] when central cholinergic receptors are blocked. Scopolamine, a muscarinic receptor antagonist, disrupts memory in both animals and humans, especially learning acquisition and short-term memory[95]. As a result, scopolamine was employed to investigate the impact of ethanolic Bixa Orellana leaves extract on memory impairment caused by cholinergic hypofunction. The memory function of rats was investigated in this study using the Elevated Plus Maze (EPM). Scopolamine was given 5 minutes before the acquisition trial to produce memory impairment, and retention was measured after 24 hours in the EPM test.

In the behavioral paradigms used in this study, both the control and vehicle groups displayed learning, as previously reported[96].

In scopolamine-induced memory impairment in rats, the impact of ethanolic leaves extract of Bixa Orellana was evaluated. On scopolamine-induced amnesia, treatment with ethanolic leaves extract of Bixa Orellana had a dose-dependent effect. Following EEBA therapy in rats, there was a substantial decrease in retention latencies and a decrease in exploration duration in EPM. All of these data demonstrated that an

ethanolic leaves extract of Bixa Orellana reduces scopolamine-induced memory impairment in rats.

Memory impairment caused by scopolamine has been linked to cholinergic hypofunction and oxidative stress in the brain.[97,98,99] The antiamnesic action of ethanolic leaves extract of Bixa Orellana was investigated in the setting of oxidative stress and cholinergic hypofunction. We measured acetylcholinesterase (AChE) activity in rat brain areas to see if the cholinergic system was involved in the antiamnesic effect. Scopolamine generated a considerable increase in AChE activity in rat brain areas, which was alleviated in a dose-dependent manner by ethanolic leaves extract of Bixa Orellana. Inhibition of AChE by ethanolic leaves extract of Bixa Orellana could resulted in an increase in ACh levels in the brain, which may explain their antiamnesic activity in the scopolamine model. As a result, cholinesterase inhibition may compensate for lower ACh levels in Alzheimer's disease brains. The researchers also looked at whether scopolamine-induced memory loss is linked to changes in oxidative stress markers. A significant imbalance between free radical generation and clearance by antioxidant mechanisms causes oxidative stress. Several studies have found a high association between memory problems in scopolamine- induced amnesia and oxidative damage patterns in people with moderate cognitive impairment. Furthermore, oxidative stress has been implicated in the pathophysiology of AD100 in several clinical trials. MDA and GSH were utilized as endogenous antioxidant and lipid peroxidation markers, respectively. MDA levels over a certain threshold indicate neuronal degeneration. GSH is the most abundant non-protein thiol in the cell and is essential for maintaining the cellular redox balance. With an increase in the formation of free radicals101, the amount of GSH decreases. MDA and GSH were calculated after behavioral investigations were

completed in the scopolamine model of amnesia. Scopolamine-treated rats had significantly higher MDA levels and lower GSH levels in the brain than control rats, indicating increased oxidative stress. The scopolamine-induced rise in MDA and decrease in GSH levels in the rat brain were dramatically reduced by an ethanolic leaves extract of Bixa Orellana. The memory- enhancing effect of ethanolic leaves extract of Bixa Orellana in the scopolamine model was related to better cholinergic function and decreased oxidative stress, according to these findings.

8.CONCLUSION:

The current study found that Scopolamine therapy resulted in severe memory impairment, as evidenced by increased acetylcholine esterase enzyme and MDA levels, as well as reduced GSH levels in several brain areas of rats. Scopolamine-induced memory impairment was greatly reduced after pretreatment with ethanolic leaves extracts of Bixa Orellana at a specific dosage and regimen. Overall, the antiamnesic action of Bixa Orellana ethanolic leaves extract can be related to its antioxidant and anti-AChE properties. After safety testing, the extract can be used in the form of nutraceuticals.

REFERENCES:

1. Thakur, V.D., Mengi, S.A., (2005), Neuropharmacological Profile of Eclipta alba Linn Hask, Journal of Ethnopharmacology, 102, pp 23- 31.

2. Rawat, M.S.M., (2011), Comparative Nootropic Effect of Evolvulus alsinoides and Convulvulus pluricaulis, International Journal of Pharma and Bioscience, 2(1), pp 616-621.

3. Sato, T., (2011), Cognitive Enhancement, its Merits and Demerits; Journal of Philosphy and Ethics in Health Care and Medicine, 5, pp 92-111.

4. Narwal, S., Saini, D.R., Kumari, K., Narwal, S., Singh, G., Negi, R.S, Sarin, R.V., (2012), Behavior and Pharmacological Animal Models for the Evaluation of Learning and Memory Condition, Indo Global Journal of Pharmaceutical Science, 2(2), pp 121-129.

5. Francis, P.T., Palmer, A.M., Snape, M., Heilcock, G.K., (1999), The Cholinergic hypothesis Of Alzheimer's Disease: a Review of Progress, Journal of Neurology, Neurosurgery & Psychiatry, 66, pp 137-147.

6. 6. Dipiro, J.T., Talbert, R.L., Yee G.C., Matzke G.R., Wells, B.G., Posey, L.M., (2008), Pharmacotherapy: A Pathophysiologic Approach, Mc Graw Hill, 7th Edition, pp 1051-1063.

7. Jain, A.K., (2007), Text book of Physiology, Avichal Publishing company, Sirmour (HP), 2(3), pp 1058-1073.

8. Chatterjee, C.C., (1997), Human physiology, 10th edition, Medical Allied Agency, Calcutta, 5(2), pp 264.

9. Guyton and Hall (2006), Text book of Medical Physiology, Saunders an imprint of Elsevier, 11, pp 714-727.

10. Bijlani, R. L., (2004), Understanding medical physiology, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 3, pp 861-864.

11. Windhorst, G.R., (1996), Comprehensive Human Physiology, Springer-Verleg Berlin Heidelberg, 1, pp 1161-1162.

12. Zola, M.S, Squire, L.R., (1990), The Neuropshychology of Memory, Parallel findings in Humans and Non Human Primates, Ann NY Acad Sci, 608, pp 434- 450.

13. Shivakumar, L., Shivaraj, G., Rao, N.V., Verma, R., (2011) Evaluation of nootropic activity of polyherbal formulation SR-105 in experimental animals, International Research Journal of Pharmacy, 2(4), pp 101-107.

14. Overview of Alzheimer's Disease, Alzheimer's Disease Facts and Figures (2010), Alzheimer's Association, Chicago, 6, pp 5.

15. Alzheimer's and Dementia, Alzheimer's Disease Facts and Figures (2013), Alzheimer's Association, Chicago, 9(2), pp 6-7.

16. Brechtold, N.C., Cotman, C.W., (1998), Evaluation in the Conceptualization of Dementia and Alzheimer's disease: GrecoRoman Period to the 1960, Neurobiology Aging, 19(3), pp 173-189.

17. Sivaraman, D., Muralidaran, P., (2010), Nootropic effect of Ipomoea aquatic Forsk in rat hippocampus, International journal of Pharm Tech Research, 2(1), pp 475-477.

18. Hashmi, M., (2009), Dementia an Anthropological Perspective, International Journal of Geriatric Psychiatry, 24, pp 207-212.

19. Gindi, S. et al. (2011), Evaluation of Nootropic Potential and in vitro Antioxidant Activity of Aqueous Extract of Roots of Asparagus racemosus in rat, International Journal of Pharmaceutical Research and Development, 3(6), pp 184-191.

20. Margineanu, D.G., (2011), A Weired Concept with Unusual fate: Nootropic Drug, Revue Des Question Scientifiques, 182(1), pp 33-52.

21. Lanni, C., Lenzken, S.C., Pascale, A. et al. (2008), Cognition Enhancer between Treating and Doping the Mind, Pharmacological Research, 57(3), 196-213.

22. Gazzaniga, M. S., (2006), The Ethical Brain: The Science of our Moral Dilemmas (P.S), Harper Perennial, pp 184.

23. Reger, B., (2006), Alzheimer disease: A brief history and avenues for current research, Journal of Young investigators, 6(2), pp 1.

24. Zaven, S., Teresa, S.R. (1996), Alzheimer's disease: Cause(s), Diagnosis, Treatment, and Care, Boca Raton, CRC, pp 32-45.

25. Maurer, K., Volk, S., Gerbaldo, H., Auguste, D., (1997), Alzheimer's disease, Lancet, 349, pp 1546-1549.

26. Graham, N.L., Emery, T., Hodges, J.R., (2004), Distinctive Cognitive Profiles in Alzheimer's disease and Subcortical Vascular Dementia; Journal of Neurology Neurosurgery & Psychiatry, 75(1), pp 61-71.

27. .Scahill, R.I., Schott, J.M., Stevens, J.M., Rossor, M.N., Fox, N.C (2002), Mapping the Evaluation of Regional Atrophy in Alzheimer's disease: unbiased Analysis of Fluid Registered Serial MRI, Proceding of the national academy of sciences USA, 99(7), pp 4703-4707.

28. Shapira, J., Schlesinger, R., Cummings, J.L., (1986), Distinguishing Dementias, American Journal of Nursing, 86(6), pp 698-702.

29. Oddo, S., Laferla, F.M., (2006), The Role of Nicotinic Acetylcholine Receptors in Alzheimer's disease, Journal of physiology, 99(2-3), pp 172-179.

30. Hardy, J., Allsop, D., (1991), Amyloid Deposition as the Central Event in the Etiology of Alzheimer's disease, trends in Pharmacological Sciences- cell 12(10), pp 383-388.

31. Polvikoski, T., (1995), Apolipoprotein E, Dementia, and Cortical Deposition of Beta-Amyloid Protein, New England Journal of medicine, 333(19), pp 1242- 1247.

32. Lacor, P.N., (2007), Aβ Oligomer Induced Aberrations in Synapse Composition, shape, and Density Provide a Molecular Basis for loss of Connectivity in Alzheimer's disease; journal of Neuroscience, 27(4), pp 796- 807.

33. Encyclopedia of Life Science (2001), Nature Publishing Group, pp 3- 4.

34. Nilson, L., Rogers, J., Potter, H., (1998), the Essential Role of Inflammation and Induced Gene Expression in the Pathogenic Pathway of Alzheimer's disease, Frontiers in Bioscience, 3, pp 436- 446.

35. Tuppo, E.E., Forman, L.J., (2001), Free Radical Oxidative Damage and Alzheimer's disease, The Journal of the American Osteopathic, Association, 101(12), pp11-15.

36. Shaik, A.S., Raja, A.E., Vijaylakshmi, M., Rao, G.D., (2010), Alzheimer's disease-Pathophysiology and Treatment, International Journal of Pharma and Biosciences, 1(2), pp 1-11.

37. Holland, A.J., Oliver, C. (1995), Down's syndrome and the links with Alzheimer's disease, Journal of Neurology, Neurosurgery &Psychiatry, 59(2), pp111-114.

38. Rockville (1996), Recognition and Initial Assessment of Alzheimer's disease and Related Dementias, Agency of Health care Policy and Research,74(4), pp 23-28.

39. Lipton, S.A., (2007), Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation, Current Drug Targets, 8(5), pp 621-632.

40. Berchtold, N.C., Cotman, C.W., (1998), Evaluation in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960, Neurobiology of aging, 19(3), pp 173-189.

41. Dementia: Quick Reference Guide (2006), National Institute for Health and Clinical Excellence London (UK), pp 435-462.

42. FDA- Approved Treatments for Alzheimer's (2012), Alzheimer's Association; pp 1.

43. Wolozin, B., Behl, C., (2000), Mechanism of Neurodegenerative Disorders, JAMA neurology, 57, pp 793-804.

44. Butterfield, D.A., Perluigi, M., Sultana, R., (2006), Oxidative Stress in Alzheimer's disease Brain: New insight from Redox Potencies, European Journal of Pharmacology, 545, pp 39-50.

45. **http://apps.worldagroforestry.org/treedb/AFTPDFS/Bixa_orellana.PDF**

46. **https://www.easyayurveda.com/2019/07/13/sinduri-bixa-orellana/**

47. M. E. A. Elias, G. Schroth, J. L. V. Macˆedo, M. S. S. Mota, and S. A. D'Angelo, "Mineral nutrition, growth and yields of annatto trees (*Bixa orellana*) in agroforestry on an Amazonian Ferralsol," *Experimental Agriculture*, vol. 38, no. 3, pp. 277 289, 2002.

48. J. Revilla, *Plantas da Amazˆonia: Oportunidades Econˆomicas e Sustent´aveis*, Manaus: Programa de Desenvolvimento Empresariale Tecnol´ogico, 2nd edition, 2001.

49. F. Oliveira, G. Akisue, and M. K. Akisue, *Farmacognosia*, Atheneu, S˜ao Paulo, Brazil, 1996.

50. J. Alonso, *Tratado de Fitof´armacos y Nutracˆeuticos*, Corpus, Ros´ario, Argentina, 2004.

51. M. P. Corrˆea, *Dicion´ario das Plantas ´ Uteis do Brasil e das Ex´oticas Cultivadas*, vol. 4,Minist´erio da Agricultura/IBDF, Rio de Janeiro, Brasil, 1978.

52. Selvi AT, Dinesh MG, Satyan RS, Chandrasekaran B, Rose C. Leaf and Seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens. J Appl Pharm Sci 2011;1 (09):116–20.

53. Viuda-martos M, Ciro-go´ mez GL, Ruiz-navajas Y, Zapatamontoya JE, Sendra E, Pe´rez-a´ lvarez JA, et al. In vitro antioxidant and antibacterial activities of extracts from annatto (Bixa orellana L.) leaves and seeds. J Food Safety 2012;32:399–406.

54. Braga FG, Bouzada MLM, Fabri RL, Matos M, Moreira FO, Scio E, et al.

Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol 2007;111:396–402.

55. Fleischer TC, Ameade EPK, Mensah MLK, Sawer IK. Antimicrobial activity of the leaves and seeds of Bixa orellana. Fitoterapia 2003;74:136–8.

56. Castello M, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L.. Ind J Exp Bio 2002;40:1378–81.

57. Stohs SJ. Safety and efficacy of Bixa orellana (achiote, annatto) leaf extracts.

Phytother Res 2014;28(7):956–60.

58. Zollo PH, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet P. Aromatic plants of tropical Central Africa Part XXXII Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour Frag J 1998;13:107–14.

59. Irobi ON, Moo-Young M, Anderson WA. Antimicrobial activity of annatto (Bixa orellana) extract. Inter J Pharmacognosy 1996;34(2):87–90.

60. Conrad OA, Dike IP, Agbara U. In vivo antioxidant assessment of two antimalarial plants – Allamamda cathartica and Bixa orellana. Asian Pac J Trop Biomed 2013;3:388–94.

61. Abayomi M, Adebayo AS, Bennett D, Porter R, Shelly- Campbell J. In vitro antioxidant activity of Bixa Orellana (Annatto) seed extract. J Appl Pharm Sci 2014;4:101–6.

62. Silva CR, Antunes LMG, Bianchi MLP. Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharm Res 2001;43:561–6.

63. Yong YK, Zakaria ZA, Kadir AA, Somchit MN, Lian GEC, Ahmad Z. Chemical constituents and antihistamine activity of Bixa orellana leaf extract. BMC Complement Altern Med 2013;13:32.

64. Zuraini A, Somchit MN, Hamid RA, Sukradi S, Fazira AJSE, Yong YK, et al.

Inhibitions of acute and chronic inflammations by Bixa orellana leaves extract. Planta Med 2007;73:76.

65. Keong YY, Arifah AK, Sukardi S, Roslida AH, Somchit MN, Zuraini A. Bixa orellana leaves extract inhibits bradykininduced inflammation through suppression of nitric oxide production. Med Princ Pract 2011;20:142–6.

66. Huama´n O, Sandoval M, Arnao I, Be´ jar E. Antiulcer effect of lyophilized hydroalcoholic extract of Bixa orellana (annatto) leaves in rats. An Fac Med 2009;70:97–102.

67. Reddy MK, Alexander-Lindo RL, Nair MG. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. J Agric Food Chem 2005;53:9268–73.

68. Antunes LMG, Pascoal LM, Bianchi MLP, Dias FL. Evaluation of the clastogenicity and anticlastogenicity of the carotenoid bixin in human lymphocyte cultures. Mut Res 2005;585:113–9.

69. Ahsan R, Islam KM, Musaddik A, Haque E. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride induced hepatotoxicity in albino rats. Global J Pharmacol 2009;3:116–22.

70. Zhai B, Clark J, Ling T, Connelly M, Medina-Bolivar F, Rivas F. Antimalarial evaluation of the chemical constituents of hairy root culture of Bixa orellana L.. Molecules 2014;19:756–66.

71. Shilpi JA, Taufiq-Ur-Rahman M, Uddin SJ, Alam MS, Sadhu SK, Seidel V. Preliminary pharmacological screening of Bixa orellana L. leaves. J Ethnopharmacol 2006;108:264–71.

72. Quanico JP, Amor EC, Perez GG. Analgesic and hypoglycemic activities of Bixa orellana, Kyllinga monocephala and Luffa acutangula. Philippine J Sci 2008;137:69–76.

73. Radhika B, Begum N, Srisailam K, Reddy VM. Diuretic activity of Bixa orellanna Linn. leaf extracts. Ind. J Nat Prod Res 2010;1(3):353–5.

74. Otero R, Nunez V, Jimenez SL, Fonnegra R, Osorio RG, Garcia ME, et al.

Snakebites and ethnobotany in the northwest region of Colombia: part II: neutralization of lethal and enzymatic effects of Bothrops atrox venom. J Ethnopharmacol 2000;71(3):505–11.

75. Ca´ ceres A, Mene´ndez H, Me´ndez E, Cohobo´ n E, Samayoa BE, Jauregui E, et al. Antigonorrhoeal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. JEthnopharmacol 1995;48:85–8.

76. Vanithakumari, G., Manonayagi, S., Padma, S., Malini, T., (1989), Antifertility effect of Bambusa arundinacea shoot extracts in male rats, Journal of Ethnopharmacology, 25, pp 173 80.

77. Kokate, C.K., (1994), Practical Pharmacognosy, 4th Ed., Vallabh Prakashan, New Delhi, pp 20-27.

78. Tota, S., Kamat, P.K., Awasthi, H., Singh, N., Raghubir, R., Nath, C., et al. (2009), Candesartan improves memory decline in mice: involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin, Behav Brain Res, 199, pp 235-40.

79. Tota, S., Kamat, P.K., Saxena, G., Hanif, K., Najmi, A.K., Nath, C., (2012), Central angiotensin converting enzyme facilitates memory impairment in intracerebroventricular streptozotocin treated rats. Behav

Brain Res, 226, pp 317-30.

80. Kulkarni, K.S., Kasture, S.B., Mengi, S.A., (2010), Efficacy study of Prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology, 42, pp 168-73.

81. Pachauri, S.D., Tota, S., Khandelwal, K., Verma, P.R., Nath, C., Hanif, K., et al., (2012), Protective effect of fruits of Morinda citrifolia L. on scopolamine induced memory impairment in mice: a behavioral, biochemical and cerebral blood flow study. Journal of Ethnopharmacology, 139, pp 34-41.

82. Tota, S., Awasthi, H., Kamat, P.K., Nath, C., Hanif, K., (2010), Protective effect of quercetin against intracerebral streptozotocin induced reduction in cerebral blood flow and impairment of memory in mice. Behaviour Brain Research, 209, pp 73-79.

83. Glowinski, J., Iversen, L.L., (1966), Regional studies of catecholamines in the rat brain. I. The disposition of [3H] norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. Journal of Neurochemistry, 13, pp 655-69.

84. Pompella, A., Visvikis, A., Paolicchi, A., DeTata, V., Casini, A.F., (2003), The changing faces of glutathione, a cellular protagonist. Biochemical Pharmacology, 66, pp 1499-503.

85. Francis, P.T., Palmer, A.M., Snape, M., Wilcock, G.K., (1999), The cholinergic hypothesis of Alzheimer's disease: a review of progress, Journal of Neurology Neurosurgery & Psychiatry, 66, pp 137-147.

86. Yamada, K., Komori, Y., Tanaka, T., Senzaki, K., Nikai, T., Sugihara, H. et al., (1999), Brain dysfunction associated with an induction of nitric oxide synthase

following an intracerebral injection of lipopolysaccharide in rats, Neuroscience, 88, pp 281-294.

87. Bartus, R.T., (2000), On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis, Experimental Neurology, 163, pp 495-529.

88. Tota, S., Kamat, P.K., Awasthi, H., Singh, N., Raghubir, R., Nath, C. et al., (2009), Candesartan improves memory decline in mice: involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin, Behaviour Brain Research, 199, pp 235-240.

89. Christensen, H., Maltby, N., Jorm, A.F., Creasey, H., Broe, G.A., (1992), Cholinergic 'blockade' as a model of the cognitive deficits in Alzheimer's disease, Brain, 115, pp 1681-1699.

90. Molchan, S.E., Martinez, R.A., Hill, J.L., Weingartner, H.J., Thompson, K., Vitiello, B. et al., (1992), Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model, Brain Research Review, 17, pp 215-226.

91. Giacobini, E., (2004), Cholinesterase inhibitors: new roles and therapeutic alternatives, Pharmacology Research, 50, pp 433-440.

92. Power, A.E., McIntyre, C.K., Litmanovich, A., McGaugh, J.L., (2003), Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors, Behaviour Pharmacology, 2003, 14, pp 207-213.

93. Sivaprakasam, K., (2006), Towards a unifying hypothesis of Alzheimer's disease: cholinergic system linked to plaques, tangles and neuroinflammation, Current Medicinal Chemistry, 13, pp 2179-2188.

94. Kakinuma, Y., Furihata, M., Akiyama, T., Arikawa, M., Handa, T., Katare, R.G. et al., (2010), Donepezil, an acetylcholinesterase inhibitor against Alzheimer's dementia, promotes angiogenesis in an ischemic hindlimb mode, Journal of Moecular & Cellular Cardiology, 48, pp 680-693.

95. Ago, Y., Koda, K., Takuma, K., Matsuda, T., (2011), Pharmacological aspects of the acetylcholinesterase inhibitor galantamine, Journal of Pharmacological Science, 11, pp 6-17.

96. Riedel, W., Hogervorst, E., Leboux, R., Verhey, F., Van Praag, H., Jolles, J., (1995), Caffeine attenuates scopolamine-induced memory impairment in humans, Psychopharmacology (Berl), 122, pp 158-168.

97. Kulkarni, K.S., Kasture, S.B., Mengi, S.A., (2010), Efficacy study of Prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats, Indian Journal of Pharmacology, 42, pp 168-173.

98. Pachauri, S.D., Tota, S., Khandelwal, K., Verma, P.R., Nath, C., Hanif, K. et al., (2012), Protective effect of fruits of Morinda citrifolia L. on scopolamine induced memory impairment in mice: a behavioral, biochemical and cerebral blood flow study, Journal of Ethnopharmacology, 139, pp 34-41.

99. Kulkarni, K.S., Kasture, S.B., Mengi, S.A., (2010), Efficacy study of Prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats, Indian Journal of Pharmacology, 42, pp168-173.

100. Joshi, H., Parle, M., (2006), Antiamnesic effects of Desmodium gangeticum in mice, Yakugaku Zasshi, 126, pp 795-804.

101. Goverdhan, P., Sravanthi, A., Mamatha, T. (2003), Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress, International Journal of Alzheimers Disease, 34, pp 28-32