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EVALUATION OF ANTI-PARKINSON'S & ANTI-ALZHEIMER'S ACTIVITIES OF MURRAYA KOENIGII & CYMBOPOGON CITRATUS FOR SYNERGISTIC ACTION ON HALOPERIDOL &ETHANOL INDUCED NEURODEGENERATIVE DISEASES IN MALE SPRAGUE-DAWELY RATS

P.Seeta[1]*, J.N.Suresh Kumar[2] ,B.Inthiyaz Begam[3] ,D.Krishnarao[3] , D.Chaitanya Lahari[3] , J.Pallavi[3] and P.Krishna Sri Priya[3]

[1]Faculty, Narasaraopeta Institute of Pharmaceutical Sciences

[2] Principal, Narasaraopeta Institute of Pharmaceutical Sciences

[3] Research scholar, Narasaraopeta Institute of Pharmaceutical Sciences

Abstract:

Background: *The aim of the present study is to evaluate the pharmacological screening of Murraya koenigii and Cymbopogon citratus for synergistic activity against neurodegenerative disorders in rodents.*

Objectives:To screen the combined effect of Murraya koenigii and Cymbopogon citratus extracts against Ethanol induced Cognitive impairment (Morris water maze (MWM) and Actophotometer) [For Learning and memory activity in Alzheimer's disease and Locomotoractivity in Parkinson's disease].

Materials and methods: *To screen the Synergistic activity of Murraya koenigii and Cymbopogon citratus extracts against haloperidol induced catatonia model in SD rats (Anti-parkinsonism/ catatonia activity), ethanol- induced cognitive impairment (learning and memory activity in Alzheimer's disease).*

Results: *For anti-parkinsonism activity scoring of Catalepsy by using motor activity by using actophotometer where as learning and memory activity was performed to evaluate the effect of Synergistic activity of Murraya koenigii and Cymbopogon citratus on alzeimer's disease by using the Morris water maze (MWM) by comparing with the control group.*

Keywords: *Murraya koenigii and Cymbopogon citratus, neurodegenerative disorders, anti-parkinsonism activity, learning and memory activity in alzeimer's disease & histopathological studies.*

Corresponding author:

P.Seeta,

Department of pharmacology,
Narasaraopeta Institute of Pharmaceutical Sciences.
Kotappakonda Road, Narasaraopeta, Andhra Pradesh -522601.
E-mail ID : Seetapendyala40964@gmail.com
Phone number:8897887579

QR CODE

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I. INTRODUCTION:

Neurodegenerative diseases (NDs) are characterized by the progressive and irreversible loss of neurons in selective regions of the brain. In afflicted individuals, symptoms typically manifest as memory loss, anxiety, and depression, which evolve to severe motor dysfunction, profound cognitive deterioration, and loss of independent function. The risk of developing a ND rises sharply with age; the number of people with a neurodegenerative disease is low at younger ages, but the prevalence of people suffering from neurodegeneration doubles every 5 years after age 65.3 AD, the most common form of NDs, currently affects about 10 percent of the population over age 65 and 47 percent of adults aged over 85 years.3 NDs typically involve a slow decline in human function that results in an eventual need for constant care and assistance with the most basic activities of daily life, consequently generating a substantial social and financial burden.[1]

Examples:

Alzheimer's disease (AD),
Parkinson's disease (PD),
Amyotrophic lateral sclerosis,
Huntington's disease, and
Friedreich's ataxia,

They are devastating age-related conditions that have become one of the primary public health concerns of recent decades. [1]

1. Parkinson's Disease (PD)

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. This disease was first described by **James Parkinson** in **1817**. It is characterised clinically by bradykinesia, resulting tremor, rigidity and postural instability. Pathological features of PD include loss of dopamine neurons in substantia nigra and presence of intracytoplasmic inclusions known as Lewy bodies in surviving dopamine neuron. It is not clear why Lewy body formation causes neuronal cell death. Among the available antiparkinson drugs, levodopa remains the most efficacious and still the mainstay of therapy. However, long term use of levodopa leads to wearing off phenomenon, on- off phenomenon, motor fluctuations and dyskinesia, which limit its further usage. Even though antiparkinson drugs are highly effective in alleviating the symptoms of Parkinsonism, but they do not give complete cure. Moreover, these drugs are often associated with frequent side effects like nausea, vomiting, depression, hallucinations, dizziness, dry mouth, sore throat, postural hypotension, diarrhea, mydraiasis, anxiety etc. The significance of many indigenous medicinal plants and their phytoconstituents in the management of Parkinsonism with minimal side effect profile arise in this context. There has been an enormous demand for further scientific development of animal models that can mimic the progressive motor impairment as in PD. One such model is Haloperidol induced catalepsy i.e., a state of akinesia with muscular rigidity in animals. It is an established model for screening the drugs for anti-parkinsonian effect. [2]

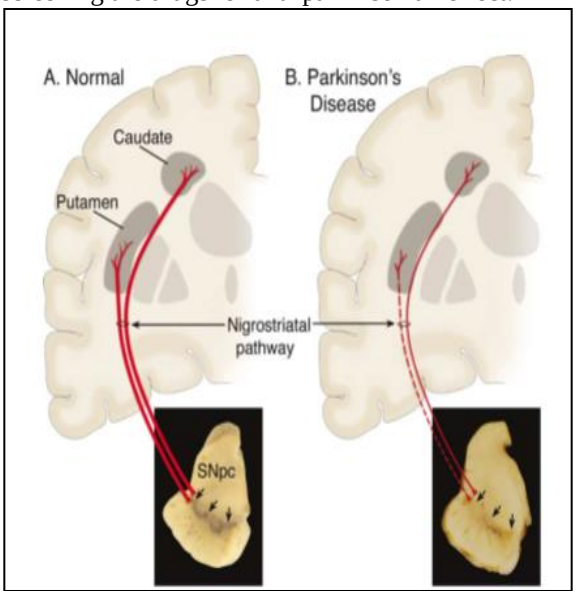

FIGURE 1: Neuropathology of Parkinson's disease [3]

(A) Schematic representation of the normal nigrostriatal pathway (in red):-It is composed of dopaminergic neurons whose cell bodies are located in the substantia nigra pars compacta (SNpc; see arrows).These neurons project (thick solid red lines) to the basal ganglia and synapse in the striatum (i.e. putamen and caudate nucleus).The photograph demonstrates the normal pigmentation of the SNpc, produced by neuromelanin within the dopaminergic neurons. [3]

(B) Schematic representation of the diseased nigrostriatal pathway (in red):- In Parkinson's disease, the nigrostriatal pathway degenerates. There is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a much more modest loss of those that project to the caudate (thin red solid line). The photograph demonstrates depigmentation (i.e., loss of dark-brown pigment neuromelanin; arrows) of the SNpc due to the marked loss of dopaminergic neurons. [3]

Causes of PD: [4]

Currently there is no known cause of understanding of why a person develops Parkinson's. There are many theories as to the causes and it is generally

thought that multiple factors are responsible. Medical experts are not yet certain what destroys the dopamine producing nerve cells or what predisposes some people to develop Parkinson's and not others . Many researchers think that the condition may be caused by a combination of genetic and environmental factors and may vary from person to person. However, Parkinson's is not an infectious disease and it is not contagious.

- **Genetics:** Several genetic changes (mutations) have been identified as increasing a person's risk of developing Parkinson's disease, although exactly how these make some people more susceptible to the condition is unclear. Parkinson's disease can run in families as a result of faulty genes being passed to a child by their parents, however, inheriting the disease in this way is rare. Recent advances in genetic studies have identified mutations in a number of pathogenic genes (SNCA, Parkin, UCHL1, DJ-1, PINK-1, LRRK2 and ATP13A2 genes) that contribute to familial forms of PD.
- **Environmental factors:** Some researchers also feel that environmental factors may increase a person's risk of developing Parkinson's disease. It has been suggested that pesticides and herbicides used in farming and traffic or industrial pollution may contribute to the condition. However, the evidence linking environmental factors to Parkinson's disease is inconclusive. The potential environmental risk factors include farming activity, pesticide exposures, well-water drinking, and history of head trauma.
- **Other causes of Parkinsonism:** Parkinsonism' is the umbrella term used to describe the symptoms of tremors, muscle rigidity and slowness of movement. Parkinson's disease is the most common type of Parkinsonism, but there are also some rarer types where a specific cause can be identified. These include Parkinsonism caused by:
  i. **Medication (drug induced Parkinsonism):** where symptoms develop after taking certain medications, such as some types of antipsychotic medication, and usually improve once the medication is stopped.
  ii. **Other progressive brain conditions:** such as progressive supranuclear palsy, multiple systems atrophy and corticobasal degeneration.
  iii. **Cerebral infarction:** where a severe stroke causes several parts of the brain to die.

Symptoms: [4]

Parkinson's entails symptoms of many types – motor and non – motor. However, not every symptom affects every PwP, & the intensity of symptoms varies across individuals. In addition to these four cardinal motor symptoms there are many others which are also considered in the diagnostic process. Often the non-motor symptoms are more challenging for the person living with Parkinson's. Non-motor symptoms such as pain, depression and problems with memory and sleep can also occur and have an impact on the day to day life of the person with Parkinson's. The Four main symptoms of Parkinson's disease affect physical movement:

- **Tremor:** The most common symptom of Parkinson's disease is the unilateral, typically resting tremor in body parts, most commonly in the upper extremities. However, this finding can spread to the other parts of the body like lips, chin, jaw and tongue during the course of the disease. It is an early symptom and is seen in about 70% of people presenting with Parkinson's. The tremor of PD is a rest tremor- the shaking occurs when the patient is not trying to use the limb, and diminishes when the limb is in use. Tremor is related to an imbalance of neurotransmitters, dopamine and acetylcholine, for this reason, tremor may be the least responsive symptom to dopamine replacement therapy. This usually begins in the hand or arm and is more likely to occur when the limb is at rest .
- **Slowness of movement (bradykinesia):** Bradykinesia can be the most disabling symptom of the condition and refers to slowness, decreased movement amplitude, and dysrhythmia. Physical movements are much slower than normal, which can make everyday tasks difficult and can result in a distinctive slow, shuffling walk with very small steps.
- **Muscles stiffness (rigidity):** Parkinson's disease can create greater tension in the tendon, leading to structural adjustment and an increase in tendon stiffness. Muscle rigidity may not be apparent to the person with Parkinson's but is felt by the medical practitioner in limb muscles when they are passively moved. Stiffness and tension in the muscles, which can make it difficult to move around and make facial expressions and can result in painful muscle cramps (dystonia).
- **Postural Instability:** Postural instability is one of the most disabling features of Parkinson's disease. Postural instability is often experienced in the late stages of PD and is a marker of disease progression. Little information is available on the role of visual inputs as an

adaptive strategy to compensate for postural instability in PD. Postural instability and gait disturbances often develop later in the progression of the condition. If a loss of postural reflexes and resulting falls occur early, it is not suggestive of typical Parkinson's. Postural instability is a disabling feature of Parkinson's disease (PD), contributing to recurrent falls and fall-related injuries. In early Parkinson's the posture may show a slight flexion of the neck or trunk with a slight lean to one side.

- **Other Symptoms:** Anosmia, Anxiety, Constipation, Depression, Fatigue, Festination of speech, Postural hypotension and Micrographia.

Progression of PD: [4]

Parkinson's is a neurological disorder that progresses slowly with time. Symptoms normally begin on one side of the body and usually spread to the other side as Parkinson's progresses. It is difficult to estimate the rate of progression as every individual with Parkinson's may experience different symptoms. Symptoms present in the earlier stages of the condition may worsen and new symptoms may appear during the course of Parkinson's. Medications help in managing the symptoms but unfortunately, aren't implicated for slowing the progression of Parkinson's.

- **Early Parkinson's:** During the initial stages of Parkinson's, the symptoms may be mild and interfere with fine motor activities like buttoning a shirt, tying shoe laces, a change in handwriting and slowed movement. Tremor if present may appear on one side of the body, starting either with the finger/hand or toe/foot.
- **Advanced Parkinson's:** As Parkinson's progresses, the symptoms that appeared earlier tend to become more pronounced and problems with balance and change in posture become evident. After years of Parkinson's, a PwP tends to walk with a stooped posture with short steps.

Symptoms of Parkinson's develop slowly and gradually progress over time. Each person is affected differently and the rate of progression varies greatly between individuals. Parkinson's doesn't directly cause people to die and it is possible to live with Parkinson's for a long time, although symptoms do get worse over time.

Pathophysiology of PD:

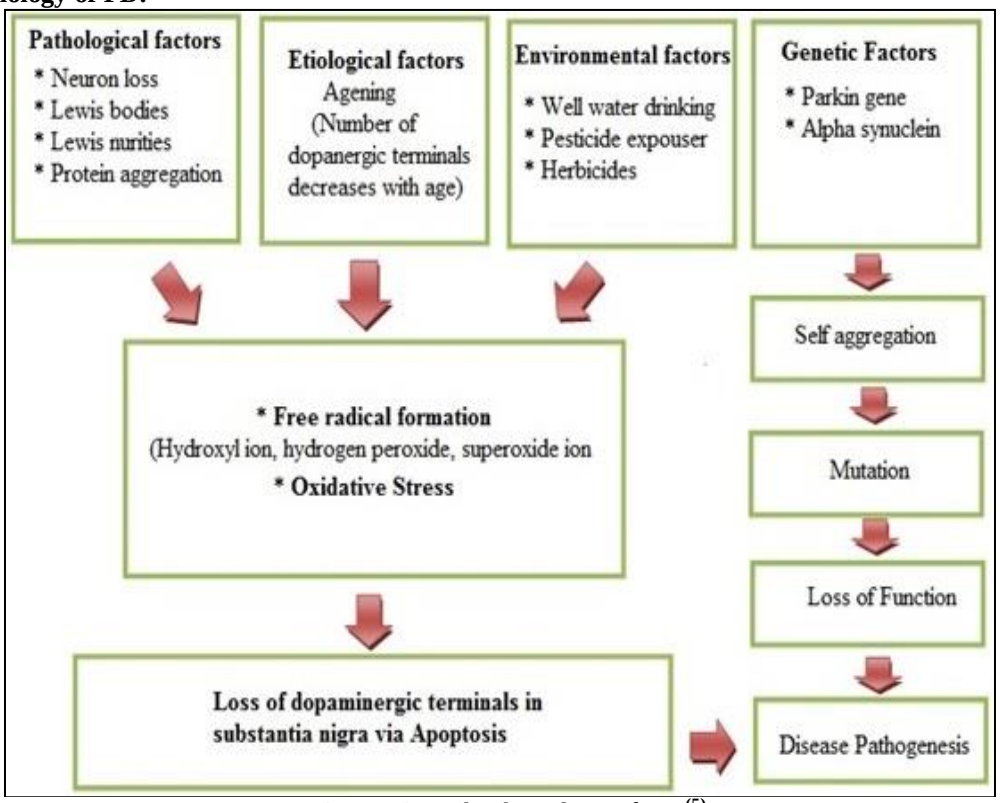

FIGURE 2: Pathophysiology of PD [5]

DRUGS USED IN PARKINSON'S

I. Drugs affecting brain dopaminergic system

1. Dopamine precursors: Levodopa (L-DOPA)
2. Peripheral decarboxylase inhibitors: Carbidopa, Benderizine
3. Dopaminergic agonist: Bromocriptine, Ropinirole, Pramipexole
4. MAO- B inhibitors: selegiline, Rasagiline
5. COMT Inhibitors: Tolcapone
6. Glutamate (NMDA) ANTAGONIST RECEPTOR: Amantadine

II. DRUGS ACTING ON BRAIN CHOLINERGIC SYSTEM:

1. Central anticholinergic: Trihexypheniyl, Procyalidine
2. Anti histamines: oxphenadrine, Promethazine

2 Alzheimer's Disease (AD)

Alois Alzheimer and Auguste D

The German psychiatrist and neuropathologist Dr. Alois Alzheimer is credited with describing for the first time a dementing condition which later became known as AD. In his landmark 1906 conference lecture and a subsequent 1907 article, Alzheimer described the case of Auguste D, a 51-year-old woman with a 'peculiar disease of the cerebral cortex,' who had presented with progressive memory and language impairment, disorientation, behavioural symptoms (hallucinations, delusions, paranoia), and psychosocial impairment.

Remarkably, many of the clinical observations and pathological findings that Alzheimer described more than a century ago continue to remain central to our understanding of AD today. [6]

Alzheimer's disease is a form of brain degeneration in which abnormal particles called neurofibrillary tangles and neuritic plaques form in the brain and destroy healthy neurons (brain cells). These abnormalities tend to settle in brain areas that control the ability to learn a new fact and remember it 30 minutes, or a day later, a skill we refer to as "memory".[7]

(OR)

Alzheimer's disease is a chronic progressive neurodegenerative disorder characterized by three primary groups of symptoms. [8]

S.No	Groups	Symptoms
1	First group (cognitive dysfunction)	Memory loss, language difficulties, and executive dysfunction (i.e. loss of higher level planning and intellectual coordination skills).
2	Second group (psychiatric symptoms)	Depression, hallucinations, delusions, agitation—collectively termed non-cognitive symptoms.
3	Third group (difficulties with performing activities of daily living)	Deemed "instrumental" for more complex activities such as driving and shopping and "basic" for dressing and eating unaided.

FIGURE-3: Schematic representation of the healthy brain and AD patient brain [9]

The symptoms of Alzheimer's disease progress from mild symptoms of memory loss to very severe dementia (figure-4). [8]

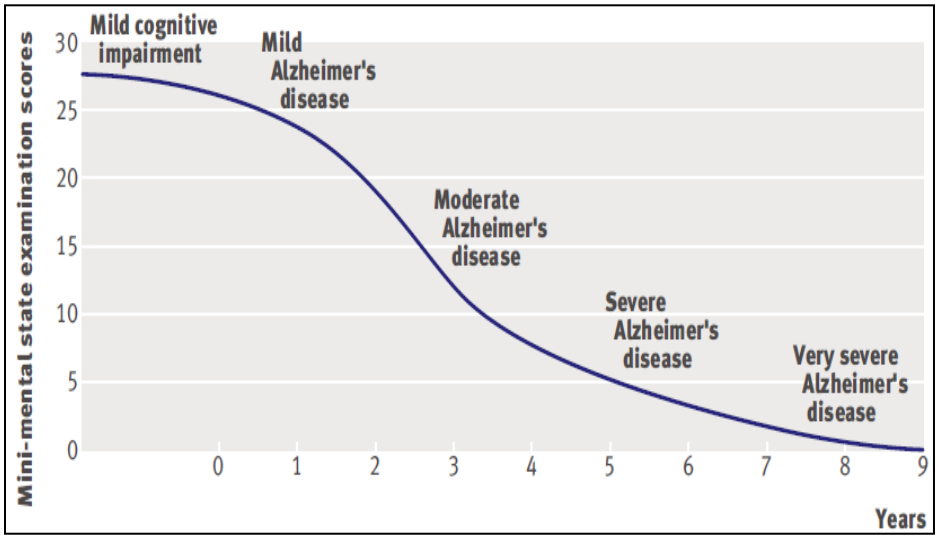

FIGURE-4: Symptom progression in Alzheimer's disease

Stages of AD: [10]

Stages	Name	Description
1	**Preclinical stage (No cognitive impairment)**	A newly defined stage reflecting current evidence that measurable biomarker changes in the brain may occur years before symptoms affecting memory, thinking or behavior can be detected by affected individuals or their physicians. While the guidelines identify these preclinical changes as an Alzheimer's stage, they do not establish diagnostic criteria that doctors can use now. Rather, they propose additional research to establish which biomarkers may best confirm that Alzheimer's-related changes are underway and how to measure them.
2	**Early stage (Very mild decline)**	Mild cognitive decline. • Difficulty in social or occupational settings • Friends or family may notice change
3	**Early stage (Mild cognitive decline)**	Mild cognitive impairment (MCI) due to Alzheimer's disease. In this stage, mild changes in memory and thinking are noticeable and can be measured on mental status tests, but are not severe enough to disrupt day-to-day life.
4	**Early stage [Moderate cognitive decline (Mild or early-stage Alzheimer's disease)]**	Probable Alzheimer's dementia. The differentiation of dementia from MCI rests of the determination of whether there is significant interference in the ability to function at work or in usual daily activities. This is a clinical judgment based on information obtained from the patient and from a knowledgeable informant.
5	**Middle stage [Moderately severe cognitive decline (Moderate or mid-stage Alzheimer's disease)]**	Moderate cognitive decline. • Difficulty performing simple tasks • May need assistance with activities of daily living such as bathing or dressing
6	**Middle stage (Moderately severe or mid-stage Alzheimer's disease)**	Severe cognitive decline.
7	**Late stage [Very severe cognitive decline (Severe or late-stage Alzheimer's disease)]**	Severe Impairment. • Supervision or complete assistance is required to complete all activities of daily living • Communication is severely impaired

SYMPTOMS: [11]

Typical early symptoms of Alzheimer's may include:

Symptoms	Description
Memory	Regularly forgetting recent events, names and faces.
Repetition	Becoming increasingly repetitive, e.g. repeating questions after a very short interval.
Misplacing things	Regularly misplacing items or putting them in odd places.
Confusion	Uncertainty about the time of day.
Disorientation	Disorientation, especially away from normal surroundings. Getting lost
Language	Problems finding the right words.
Mood and behavior	Some people become disinterested in what's happening around them, become irritable, or lose confidence.
Memory and thinking skills	People will find that their ability to remember, think and make decisions worsens
Communication	Communication and language become more difficult.
Behaviour	A person's behavior may change and some people can become sad or depressed. Anger and agitation become more common and people may develop anxieties or phobias
Hallucinations	People may experience hallucinations, where they may see things or people that aren't there.
Restlessness	Problems with sleeping and restlessness at night often occur.
Unsteadiness	People may become increasingly unsteady on their feet and fall more often
Daily activities	People gradually require more help with daily activities like dressing, toileting and eating.

Causes ofAD : [12]

While scientists know that Alzheimer's disease involves the failure of nerve cells, it's still Unknown why this happens. However, they have identified certain risk factors that increase the likelihood of developing Alzheimer's.

- **Age:** The greatest known risk factor for Alzheimer's is increasing age. Most individuals with the disease are 65 and older. One in nine people in this age group and nearly one-third of people age 85 and older have Alzheimer's.
- **Family history:** Another risk factor is family history. Research has shown that those who have a parent, brother or sister with Alzheimer's are more likely to develop the disease than individuals who do not. The risk increases if more than one family member has the illness.
- **Genetics:** Researchers have found several genes that increase the risk of Alzheimer's. APOE-e4 is the first risk gene identified and remains the one with strongest impact. Other common forms of the APOE gene are APOE-e2 and APOE-e3. Everyone inherits a copy of some form of APOE from each parent. Those who inherit one copy of APOE-e4 have an increased risk of developing Alzheimer's; those who inherit two copies have an even higher risk, but not a certainty.

Pathophysiology of AD : [13]

AD is characterized by progressive loss of brain tissue,extracellular plaques of β- amyloid protein, intracellular neurofibrillary tangles and neuronal degeneration.

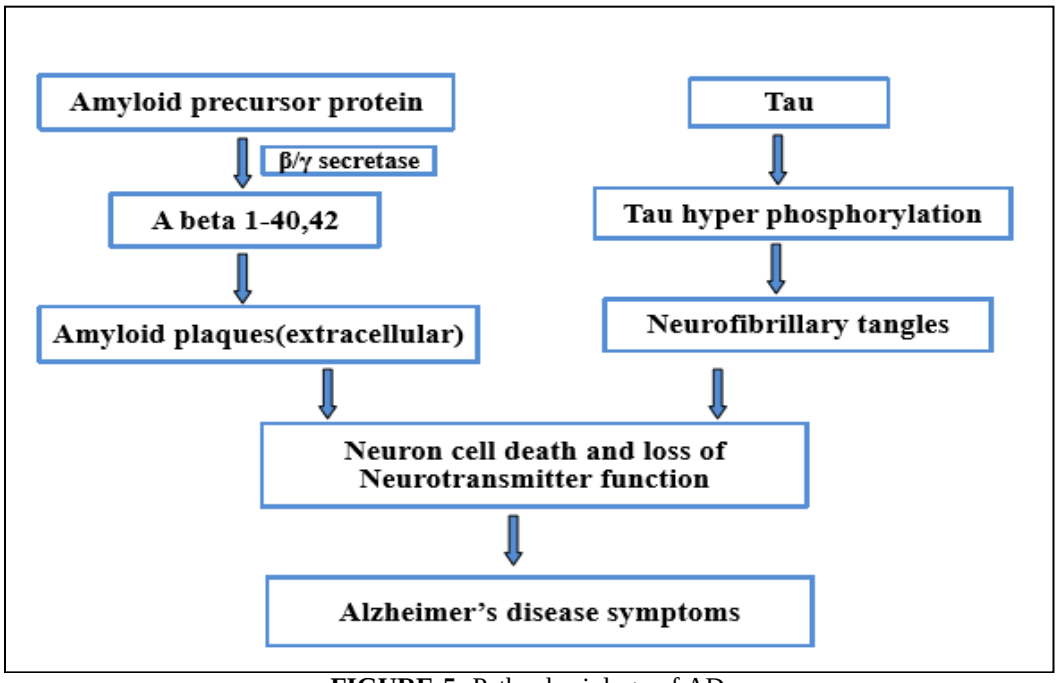

FIGURE-5: Pathophysiology of AD

DRUGS USED IN AD[14]

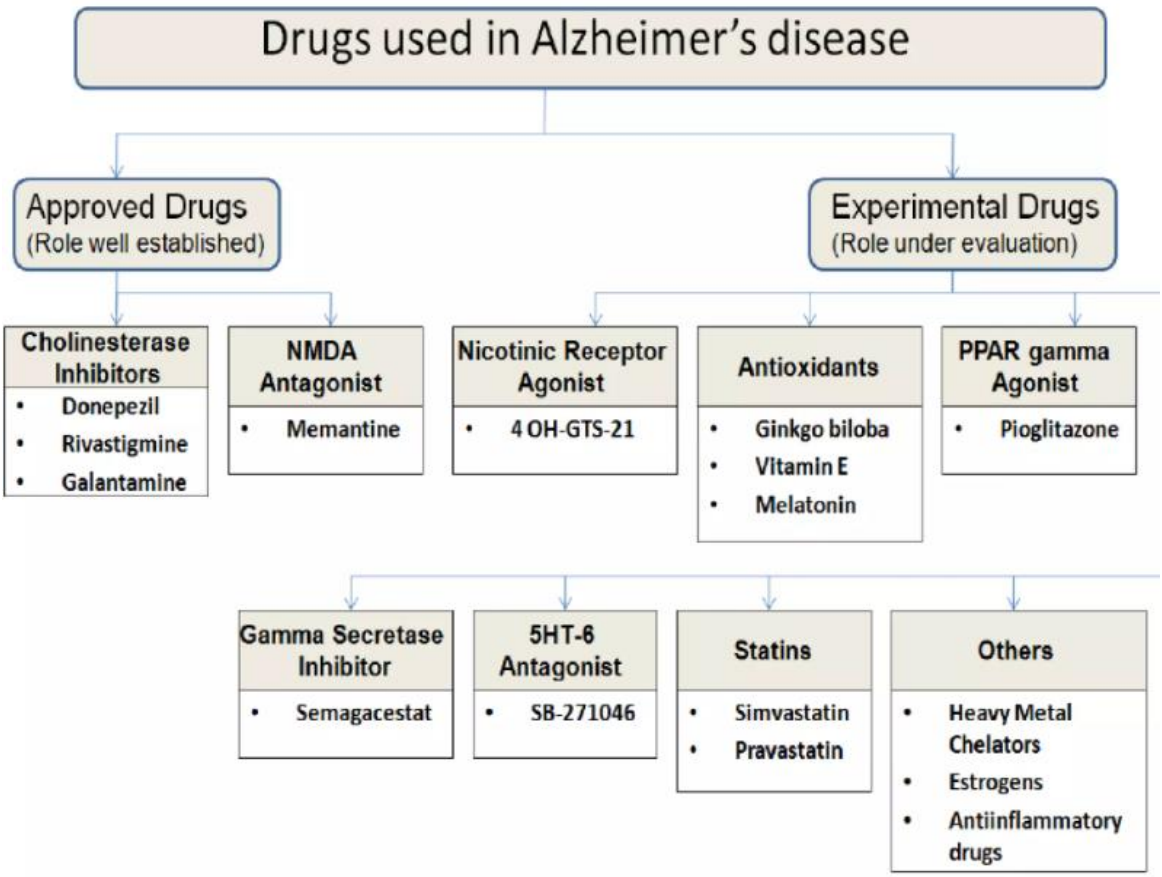

FIGURE-6: Drugs used in ad

II. MATERIALS AND METHODS:

II. 1. INVESTIGATIONS OF ANTIPARKINSONIAN EFFECT BY USING ACTOPHOTOMETER

1.1 Haloperidol Induced Catatonia Model in SD Rats:

Haloperidol {4-(4-chlorophenyl)-1-(4-(4-flurophenyl)-4-oxobutyl)-4-piperidinol} is the widely used antipsychotic drug and it shares some structural similarity with 1- methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). MPTP is identified as the toxic agent present in heroin and responsible for neurodegenerative condition similar to Parkinson's disease. MPTP is commonly used to induce Parkinsonism in experimental animals. Haloperidol is metabolized in liver, it undergoes oxidation to the pyridinium metabolite, 4-(4-chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)-pyridinium (HPP+) which shares some structural similarity and toxic actions with pyridinium metabolite of MPTP 1-methyl-4-phenylpyridine (MPP+). This suggests that HPP+ might produce neurological effects similar to MPTP. Therefore, in the present study haloperidol is used to induce Parkinsonism in rats. [26] Haloperidol is known to produce extrapyramidal side effects in man. These effects, such as akinesia, rigidity and tremors, are called Parkinson's-like because in Parkinson's disease the major clinical symptoms include difficulty to move and change posture (akinesia and rigidity) and tremors. These effects of antipsychotic drugs are due to excessive blockade of dopamine receptors in the extrapyramidal motor system. Therefore, butyrophenones [haloperidol (or) trifluperidol] are commonly used to produce Parkinson's-like extrapyramidal symptoms in laboratory animals and to study anti-parkinsonian drugs. [15]

➢ **Selection of Dose and Treatment Period:**

The anti-parkinsonism activity of the aqueous and ethanolic leaf extracts of *Murraya koenigii* and *Cymbopogon citratus* was investigated using the haloperidol induced catatonia method [Haloperidol is widely used to induce Parkinsonism like condition in the dose 0.5 to 4 mg/kg daily for a week in rats]. [16] The test animals were randomly chosen and divided into five groups having five rats in each as follows:

Group I: Inducing group - Haloperidol (4mg/kg, p.o once/day x 1 week).

Group II: Standard group - Syndopa plus [28] [(Levodopa+ carbidopa) (10mg/kg, p.o, once/day x 1 week)] +Haloperidol.

Group III: Test-I – 100mg/kg of aqueous extract of *Murraya koenigii* leaf [P.O x1week] +Haloperidol.

Group IV: Test –II - 200mg/kg of alcoholic extract of *Cymbopogon citratus* leaf [P.Ox1week] +Haloperidol

Group V: Test – III - 70mg/kg of *Murraya koenigii* leaf extract& 90mg/kg of *Cymbopogon citratus* leaf extract+ Haloperidol [P.O x1week]

All the treatment group animals received respective control, standard and test treatment 30 minutes prior to the haloperidol administration for 7 days of experimental period.

1.2 Locomotor Activity Using Actophotometer: [17]

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photo electric cells which are converted in circuit with a counter. When a beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could be either circular or square area in which the animal moves. Calculate the % decrease in motor activity (or) % activity change (Before - After 30 minutes / before × 100).

➢ **Evaluation parameters:**

i. No. of counts / 5 min i.e. after 30 min.
ii. % decrease in motor activity (or) % activity change (Before - After 30 minutes / before × 100).

Fig: 7. Actophotometer

II. 2. INVESTIGATION OF LEARNING AND MEMORY ACTIVITY IN ALZHEIMER'S MODEL BY USING MORRIS WATER MAZE (MWM)

2.1. Morris Water Maze (MWM):

To assess hipocampal dependent spatial learning and memory, all rats were trained in a standard Morris water maze task (Morris et al., 1982; Stackman et al., 2002).[18] Maze consisted of large circular pool (75cm &30cm) filled with water at a depth of 20cm. The pool was divided into four quadrants. A circular platform was placed at the centre of one quadrant. The rats performed four trials per day for four consecutive days. In the swimming trials, each

individual rat was released gently into the water at a randomly chosen quadrant. The rats swim and learned how to find the hidden platform within 60 s. After reaching the platform rat was allowed to stay on the platform for 15 s and was then taken back into the cage. The rats were placed on the platform by hand for 15 s, if they could not escape to the platform within 60 s by themselves, and their escape latency was accepted as 60 s. During the inter-trial intervals, animals were kept in a dry home cage for 60 s. The time to reach the platform (latency) was recorded. 24h after the last day of training, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60 s trial. [19]

TABLE NO.2: The sequence of trials during the study period of MWM test[20]

1st day	2nd day	3rd day	4th day
Q1	Q2	Q3	Q4
Q2	Q3	Q4	Q1
Q3	Q4	Q1	Q2
Q4	Q1	Q2	Q3

FIGURE-8: MWM

- **Evaluation parameters:**
  Transfer latency in sec

2.2 Ethanol- Induced Cognitive Impairment: [20]

Ethanol is neurotoxin that able to alter behavioural and cognitive performance in experimental animals in addition to humans. It mainly impairs hippocampus-dependent learning and memory functions. The mechanism of ethanol-induced neurotoxicity is not well understood. Several studies show that free-radical mediated oxidative stress play an imperative role. The brain is extremely susceptible to oxidative stress due to high level of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O_2) in relatively small mass and in conjunction with low antioxidant activities. Furthermore, certain regions of the central nervous system (CNS), especially hippocampus and cerebellum, may be more sensitive to oxidative stress because of their low endogenous antioxidant, in relation to other brain regions. Study showed that acetaldehyde dehydrogenase is responsible for the generation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from oxidation of ethanol to acetate. It has been confirmed that ethanol induces the synthesis of CYP2E1 that lead to oxidative stress. It also increases the ratio of NADH/NAD, responsible for reduction of ferric ion (Fe3+) to ferrous ion (Fe2+)) which causes lipid peroxidation by generating hydroxyl radical.

➢ **Selection of Dose and Treatment Period:**

The learning and memory enhancing activity of the aqueous and ethanolic fruit extracts of *Terminalia chebula* was investigated using the ethanol- induced cognitive impairment [Ethanol (60%) is used to induce dementia like condition in the dose 2.5 mg/kg administered i.p for 15 days). [31] The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group- Ethanol (2.5 mg/kg was administered i.p for 15 days).

Group II: Standard Group -Donepezil hydrochloride [32] (2.5 mg/kg was administered orally for 15days) + Ethanol.

Group III: Test-I -Aqueous fruit extract of *Terminalia chebula* [TCAE- 100mg/kg was administered orally for 15days) + Ethanol.

Group IV: Test -II -Ethanolic fruit extract of *Terminalia chebula* [TCEE- 100mg/kg was administered orally for 15 days) + Ethanol.

2.3 Diazepam Induced Amnesia:

Diazepam 1mg/kg, i.p was administered to rats and TL was noted after 45 min of injection on 8th day and after 24hrs. Extracts and standard Donepezil hydrochloride were administered for successive 8 days. After 60 min of administration of the last dose on 8th day, Diazepam 1mg/kg i.p was administered. TL was noted after 45 min administration of diazepam and after 24 hrs. [21]

➢ **SELECTION OF DOSE AND TREATMENT PERIOD:**

The learning and memory enhancing activity of the aqueous and Ethanolic fruit extracts of *Terminalia chebula* was investigated using the [Diazepam is used to induce amnesia like condition in the dose 1 mg/kg administered i.p for 8 days). [21] The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group-Diazepam (1mg/kg was administered i.p for 8 days).

Group II: Standard Group-Donepezil hydrochloride [32] (2.5 mg/kg was administered orally for 8days).

Group III: Test-I- Aqueous fruit extract of *Terminalia chebula* [TCAE- 100mg/kg was administered orally for 8days).

Group IV: Test –II- Ethanolic fruit extract of *Terminalia chebula* [TCEE- 100mg/kg was administered orally for 8 days).

III RESULTS AND DISCUSSION:

III.1 Effect of Leaf Extracts of *Murraya koenigii* and *Cymbopogon citratus* on Behavioural Parameters

(Locomotor Activity):

Actophotometer: Animals treated with haloperidol (4 mg/kg, P.O) alone for 7 days showed a decrease of locomotor activity [no. of counts / 5 min i.e. after 30 min] on 1st day,4th day &7th day and also reduction in the %change activity when compared to other groups.

Table - 1: Effect of leaf extracts of *Murraya koenigii* and *Cymbopogon citratus* on locomotor activity

S.No.	Group	Treatment	Locomotor activity [no. of counts / 5 min i.e. after 30 min]								
			1 ST DAY			4TH DAY			7TH DAY		
			Before	After	% Change activity	Before	After	% Change activity	Before	After	% Change activity
1.	I	**Haloperidol**	266 ± 21.24	150.2 ± 12.43	**43.5%**	138.8 ± 5.46	74.4 ± 4.95	**46.39%**	87.0 ± 2.67	49.4 ± 5.04	**43.2%**
2.	II	**Standard + haloperidol**	159 ± 6.40	75.0 ± 12.45	**52.83%**	27.4 ± 2.45	11.4 ± 2.44	**58.39%**	27.6 ± 1.12	8.0 ± 1.52	**71%**
3.	III	**MK+ haloperidol**	172.6 ± 6.39	86.6 ± 12.92	**49.82%**	32.4 ± 0.93	21.2 ± 2.33	**34.56%**	33.0 ± 1.30	21.6 ± 2.25	**34.5%**
4.	IV	**CC+ haloperidol**	165.2 ± 4.81	82.6 ± 12.96	**50%**	28.4 ± 0.98	17.0 ± 2.47	**40.1%**	29.0 ± 1.30	12.2 ± 1.93	**57.9%**
5.	V	**MK+CC haloperidol**	174± 5.25	86.8± 10.95	**49.88%**	30.5±1	17.2± 3.0	**57.3%**	34.0±2	22.4	**65.8%**

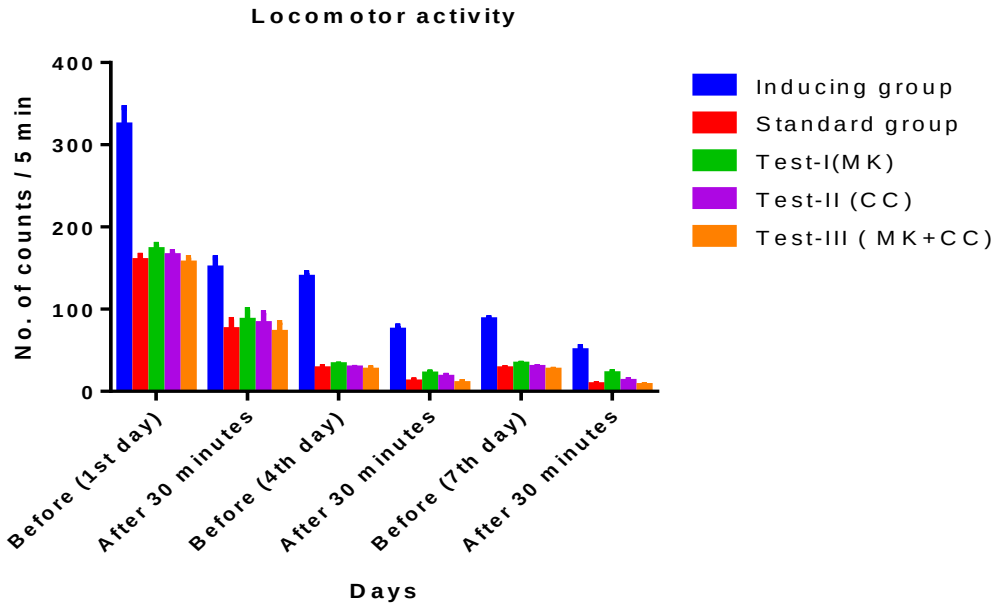

Fig: 9

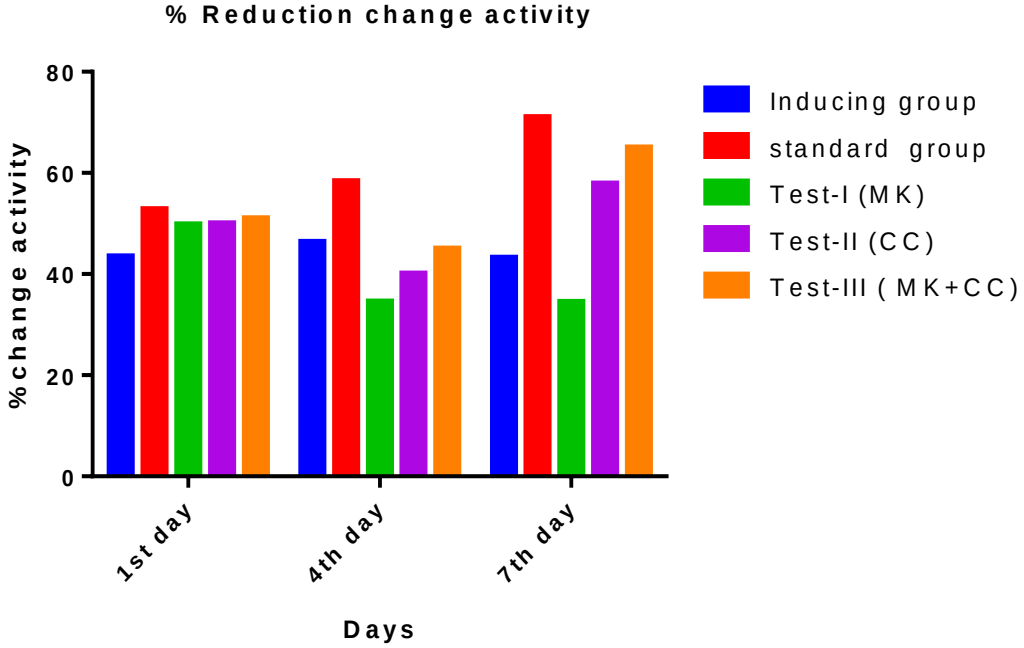

Fig: 10

Effect of leaf extracts of *Murraya koenigii* and *Cymbopogon citratus* on behavioural Parameters (motor activity). Values are expressed as mean ± SE, $p < 0.01$ *vs.* control, n = 5 animals.

III.2 & III.3 Effect of leaf Extracts of *Murraya koenigii* and *Cymbopogon citratus* on Behavioural Parameters i.e. MWM:

Animals treated with ethanol [2.5 mg/kg] alone for15 days showed an increase in transfer latency in seconds on 1^{st},7^{th}& 15^{th} days as well as diazepam [1mg/kg] alone for 8 days showed an increase in transfer latency in seconds on 8^{th} day & after 24 hrs i.e. 9^{th} day.

Table –3. Effect of leaf extracts of *Murraya koenigii* and *Cymbopogon citratus* on transfer latency (ethanol- induced cognitive impairment)

S.No.	Group	Treatment	Transfer latency (In seconds)		
			1st DAY	7th DAY	15th DAY
1.	I	**Ethanol**	24.2 ±2.35	27.6±3.89	27.8±4.04
2.	II	**Standard + ethanol**	5.6±1.47	4.6±1.21	2.6±0.68
3.	III	**MK+ ethanol**	7.4±1.69	6.6±2.42	3.2±0.97
4.	IV	**CC+ ethanol**	5.8±1.62	4.4±1.54	2.8±0.66
5	V	**CC + MK + Ethanol**	9.5±1.56	8.2±2.1	3.8±0.77

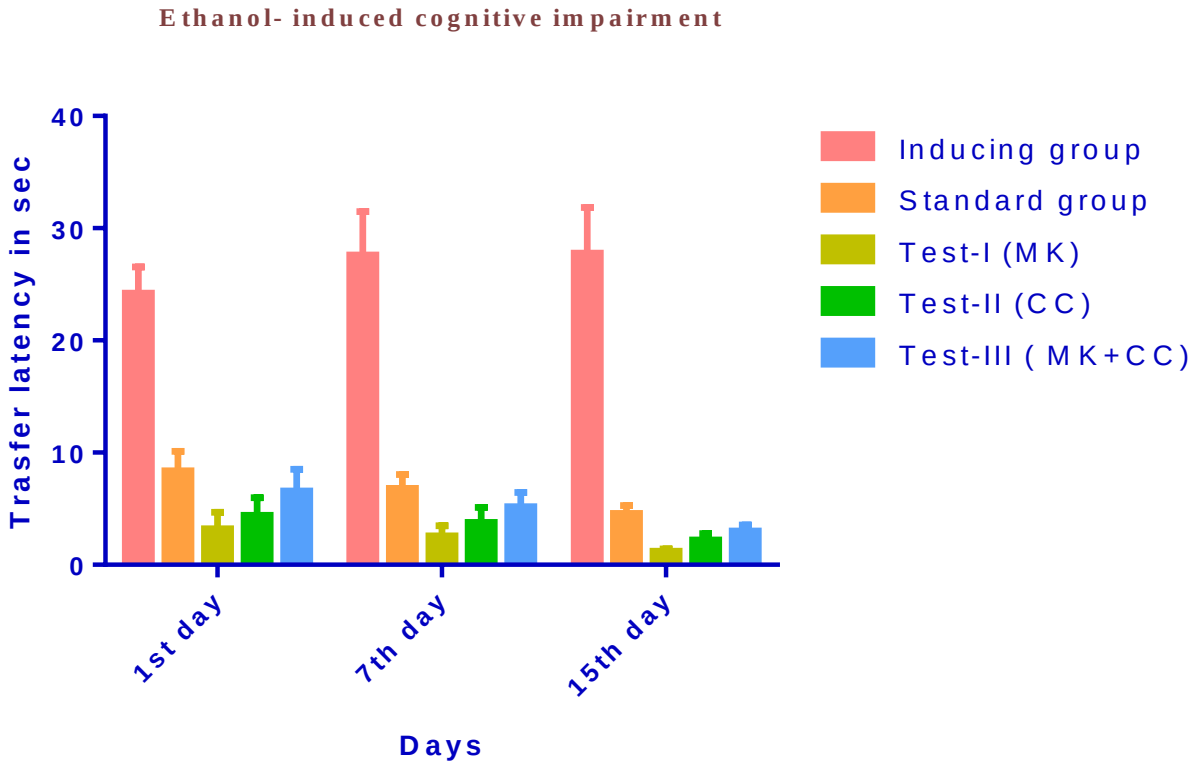

Fig: 11

Effect of **leaf Extracts of *Murraya koenigii* and *Cymbopogon citratus*** on ethanol- induced cognitive impairment. Values are expressed as mean ± SE, $p < 0.01$ vs. control (n = 5 animals).

IV. SUMMARY AND CONCLUSION:

- In the present investigation, *Murraya koeingii and Cymbopogon citrates* posses the presence of alkaloids, carbohydrates, phenols, saponins, terpenoids, phenols, tannins, proteins, amino acids and glycosides.
- Individual and Synergitic activity of *Murraya koeingii and Cymbopogon citrates* leaves extract showed anti-cholinergic mechanism i.e. anti-parkinsonism effect, at an effective dose of 100 mg/kg against haloperidol induced parkinsonian symptoms.
- Individual and Synergitic activity of *Murraya koeingii and Cymbopogon citrates* leaves extract showed comparatively significant effect exerted to standard drug Syndopa in the finding of locomotor activity. Locomotor activite in anti-alzheimer's activity was recorded after

administration of haloperidol at different time intervals and graphs were ploted according to the results obtained.

- Individual and Synergitic activity of *Murraya koeingii and Cymbopogon citrates* leaves extract showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol-induced cognitive impairment in rats.
- Therefore finally suggests Synergitic activity of *Murraya koeingii and Cymbopogon citrates* leaves extract showed same effects exerted to the standard drugs than individual leaves extract against . And further studies are needed for the human use.

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