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# COGNITIVE ENHANCING ACTIVITY OF HERBAL EXTRACT IN SCOPOLAMINE INDUCED AMNESIA IN EXPERIMENTAL ANIMALS

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**Abstract:**

*In 1901 German psychiatrist Alois Alzheimer identified the first case what became known as Alzheimer disease in a fifty year old women Augeste D .Alzheimer follow her until she died in 1906, it's an first case reported publicaly. Alzheimer disease is a neurodegenerative disorder produces impairment of cognitive abilities that is gradual in onset but relentness in progression.AD refers to dementia that does not have an antecedent cause, such as stroke, brain trauma or alcohol. Its prevalence rises sharply with age, from about 5% at 65 to 90% or more at 95.*

*AD is often confused with normal aging and dementia. Severe memory loss, characteristic of AD, is not a symptom of normal aging. Healthy aging may involve the gradual loss of hair, weight, height and muscle mass. Skin may become more fragile and bone density can be lost. A decrease in hearing and vision may occur, as well as a decrease in metabolic rate. It is common to have a slight decline in memory, such as slower recall of information, however cognitive decline that impacts daily life is not a normal part of the aging process . Dementia is defined as the significant loss of cognitive abilities severe enough to interfere with social functioning . It can result from various diseases that cause damage to brain cells. There are many different types of dementia, each with its own cause and symptoms.Artemisia Absinthium Is One Of The Most Commonly Use In Different Medical Conditions Has Been Documented. Traditionally, Artemisia Absinthium Are Beneficial In The Treatment Of Various Diseases . As No Reports Are Available On The Possible Cognitive Enhancing Activity Of Artemisia Absinthium.The Present Work Was Carried Out To Cognitive Enhancing Activity Of Artemisia Absinthium By Scopolamine Induced Amnesia Test Drug Artemisia Absinthium ( Low Dose) And Artemisia Absinthium (High Dose) Have Shown Result In Comparison To Standard Drug Piracetam And Control Drug. Thus Study Has Provided Documentary Evidence For Cognitive Enhancing Property Of Artemisia Absinthium For Its Activity.*

**Keywords:** *Alzheimer Disease, Artemisia Absinthium, Scopolamine, Amnesia, Piracetam.*

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## I.INTRODUCTION:

### ALZHEIMER'S DISEASE

In 1901 German psychiatrist Alois Alzheimer identified the first case what became known as Alzheimer disease in a fifty year old women Augeste D .Alzheimer follow her until she died in 1906, it's an first case reported publicaly. Alzheimer disease is a neurodegenerative disorder produces impairment of cognitive abilities that is gradual in onset but relentness in progression.AD refers to dementia that does not have an antecedent cause, such as stroke, brain trauma or alcohol. Its prevalence rises sharply with age, from about 5% at 65 to 90% or more at 95. Until recently, age-related dementia was considered to result from the steady loss of neurons that normally goes on throughout life, possibly accelerated by a failing blood supply associated with atherosclerosis. Impairment of short-term memory usually is the first clinical feature, whereas retrieval of distant memories is preserved relatively well into the course of the of the disease. As the condition progresses, additional cognitive abilities are impaired, among them the ability to calculate, exercise visuospatial skills, and use common objects and tools ( ideomotor apraxia). The level of arousal or alertness of the patient is not affected until the condition is very advanced, nor is there motor weakness, although muscular contractures are an almost universal feature of advanced stages of the disease. Death, most often from a complication of immobility such as pneumonia or pulmonary embolism, usually ensues within 6 to 12 years of onset.

AD is often confused with normal aging and dementia. Severe memory loss, characteristic of AD, is not a symptom of normal aging. Healthy aging may involve the gradual loss of hair, weight, height and muscle mass. Skin may become more fragile and bone density can be lost. A decrease in hearing and vision may occur, as well as a decrease in metabolic rate. It is common to have a slight decline in memory, such as slower recall of information, however cognitive decline that impacts daily life is not a normal part of the aging process . Dementia is defined as the significant loss of cognitive abilities severe enough to interfere with social functioning . It can result from various diseases that cause damage to brain cells. There are many different types of dementia, each with its own cause and symptoms. For example, vascular dementia is caused by decreased blood flow to a part of the brain, as caused by a stroke. Dementia may also be present in patients with Parkinson's disease and hydrocephalus. AD is the most common form of dementia, caused by the build-up of beta amyloid plaques in the brain.

### CAUSES OF ALZHEIMER DISEASE:

Alzhiemer disease is caused by a combination of genetic, lifestyle and environmental factors that Affect the brain over time. Less than 5 percent of the time, Alzhiemer's is caused by specific genetic changes that virtually guarantee a person will develop the disease.

Plaques **:** These clumps of a protein called beta-amyloid may damage and destroy brain cells in several ways, including interfering with cell-to-cell communication. Although the ultimate cause of brain-cell death in Alzheimer's isn't known, the collection of beta-amyloid on the outside of brain cells is a prime suspect.

Tangles : Brain cells depend on an internal support and transport system to carry nutrients and other essential materials throughout their long extensions. This system requires the normal structure and functioning of a protein called tau.

### CLINICAL FEATURES OF ALZHEIMER DISEASE :

**MILD ALZHIEMER DISEASE -**

- Memory loss
- Poor judgment leading to bad decisions
- Loss of spontaneity and sense of initiative
- Taking longer to complete normal daily tasks
- Repeating questions
- Trouble handling money and paying bills
- Wandering and getting lost
- Losing things or misplacing them in odd places
- Mood and personality changes

**MODERATE ALZHIEMER DISEASE-**

- Increased memory loss and confusion
- Inability to learn new things
- Difficulty with language and problems with reading, writing, and working with numbers
- Difficulty organizing thoughts and thinking logically
- Shortened attention span
- Problems coping with new situations
- Difficulty carrying out multistep tasks, such as getting dressed
- Problems recognizing family and friends
- Hallucinations, delusions, and paranoia
- Impulsive behavior such as undressing at inappropriate times or places or using vulgar language
- Inappropriate outbursts of anger.

- Restlessness, agitation, anxiety, tearfulness, wandering—especially in the late afternoon or evening

**SEVERE ALZHIEMER DISEASE -**

- Inability to communicate
- Weight loss
- Seizures
- Skin infections
- Difficulty swallowing
- Groaning, moaning, or grunting

**PATHOPHYSIOLOGY:**

Pathophysiology of Alzheimer disease is mainly related with brain shrinkage and neuronal loss from particular area mainly Hippocampus and Basal forebrain. Loss of cholinergic neurons in the hippocampus and frontal cortex is a feature of the disease, and is thought to underlie the cognitive deficit and loss of short-term memory that occur in AD. The pathological hallmarks of AD are senile plaques, which are spherical accumulations of the protein β-amyloid accompanied by degenerating neuronal process, and neuro-fibrillary tangles, comprising filaments of a phosphorylated form of a microtubule-associated protein Tau and other proteins. Although small number's of tangles are found in normal person. Severity of disease is directly proportional to presence of tangles. Amyloid protein is produce from the amyloid precursor protein (APP). The genetic analysis of certain, relatively rare, types of familial AD, in which mutations of the APP gene, or of other genes that control amyloid processing, have been discovered. The APP genes resides on chromosome 21, which is duplicated in Down's syndrome, in which early AD-like dementia occurs in association with over expression of APP.

**DIAGNOSIS:**

Alzheimer disease is diagnosed clinically from the patient history, collateral history from relatives and clinical observations, based on the presence of neurological and neuropsychological features and the absence of alternative conditions. Advanced medical imaging with medical computed tomography (CT) or magnetic resonance imaging (MRI), and with single photon emission computed tomography (SPECT) or positron emission tomography can be used to cerebral pathology Moreover, it may predict conversion from prodromal stages ( mild cognitive impairment) to Alzheimer's disease.

Neurological test such as mini-mental state examination are widely used to evaluate the cognitive impairments neede for diagnosis. Interviews with family members, relatives and caregiver's are also utilized in the assessment of the disease. Another recent objective marker of the disease is the analysis of depression, it is an early symptoms of Alzheimer's.

Patients with AD having decreased glutamate (Glu) as well as decreased Glu/creatine (Cr), Glu/Myo-inositol(ml), Glu/N-acetiaspartate(NAA), and NAA/Cr ratios compared to normsl patients. Both decreased NAA/Cr and decreased hippocampal glutamate may be early indicator of AD.

**TREATMENT:**

Therapeutic approaches for the Alzheimer's disease are following.

Cholinesterase Inhibitor's : A major approach to the treatment of AD has involved attempts to augment the cholinergic function of the brain. An early approach was the use of precursors of acetylcholine synthesis, such as choline chloride and phosphatidyl choline (lecthin). A somewhat more successful strategy has been the use if inhibitors of acetylcholine (AchE), the catabolic enzyme for acetylcholine.

Tacrine, the first drug approved for treating AD, was investigated on the basis that enhancement of cholinergic transmission might compensate for the cholinergic deficit. Tacrine has to be given four times daily and produces cholinergic side effects such as nausea and abdominal cramps, as well as hepatotoxicity in some patients, so it is far from an ideal drug. Later compounds, which also have limited efficacy but are more effective than tacrine in improving quality of life, include donepezil, rivastigmine and galantamine.

Four inhibitors of AchE currently are approved by the FDA for treatment of Alzhiemer's disease tacrine (1,2,3,4-tetrahydra-9-aminoacridine; COGNEX), donepzil (ARICEPT), rivastigmine (EXCELON), and galantamine (RAZADYNE).

Tacrine is a potent centrally acting inhibitor of AchE, Rivastigine and galantamine are dosed twice daily and produce a similar degree of cognitive improvement. Adverse effects associated with donepezil, rivastigmine, galantamine are similar in character but generally less frequent and less severe than those observed with tacrine; they include nause, diarrhea, vomiting, and insomnia. Donepezil, rivastigmine, and galantaine are not associated with the hepatotoxicity that limits the use of tacrine.

Glutamate (NMDA) Antagonist: Memantine is the NMDA receptor antagonist, related to amantadine has been found to slow the functional decline in moderate to severe AD but benefit in milder disease are unclear. It appears to block excitotoxicity of the

transmitter glutamatein a noncompetitive and use depends manner. Memantine is the better tolerated than the Anti-AchEs used in AD. Side effects are constipation, tiredness, headache and drowsiness.

**IMPORTANCE OF HERBAL DRUGS:**

The world health organization (WHO) has recently defined traditional medicine (including herbal drug) as comprising therapeutic practices that have been in existence, often for hundreds of year, before the development and spread of modern medicine and are still use today. Traditional medicines is the synthesis of therapeutic experience of generation of practicing physicians if indigenous systems of medicines. The tarditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The classical Indian texts include Rigveda, Atherveda, Charak sammhita and suushrita samhita. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilization and scientific heritage.

Herbal medicine are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptibility and lesser side effects the chemical constituents presents in them are a part of physiological functions of living flora and hence they believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age related disease namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicines or only palliative therapy is available.

**ROLE OF ANTIOXIDANT:**

Many clinical studies have reported strong evidence that oxidative stress is involved in the pathogenesis of Alzheimer's disease. The oxygen free radicals are implicated in the process of age related decline in the cognitive performance may be responsible for the development of Alzheimer's disease in elderly person's an increased oxidation of lipids and proteins and deoxyribinuleic acid , alterations in mitochondrial function and a possible role of amyloid beta and it's precursor protein in oxidative reaction in experimental models of AD are demonstrated. Moreover, strong evidence supporting in involvement of oxidative damage in neurodegeneration disease has been suggested by various clinical studies. Antioxidant enzymes such glutathione peroxidase, superoxide dismutase as well as catalase and glutathione reductase and ascorbate are involved in the reduction of oxidative stress. Antioxidant enzymes display the reduced activities in the affected brain region of patient's of Alzheimer's disease. Moreover reduction in the level of intracellular oxidized protein under these conditions has been associated with the improvement of cognitive and/or psychomotor functions. Augmentation of endogenous antioxidants by therapeutic substances has recently evoked scientific interest because any such property of therapeutic agent can be expected to cause significant improvement in the endogenous defence against oxidative stress. These agents also reduce the oxidative damage and promote a funtional recovery in degenerative disorders. So above all evidence, may prove the importance of antioxidants in neurodegenerative disease.

## 2. MATERIALS AND METHODS:

**MATERIALS:**

**COLLECTION AND AUTHENTIFICATION OF PLANT MATERIAL:**

Leaves powder of Artemisia absinthium was collected from the VHCA Ayurveda LLP. Authentification of plant on the basis of pharmacognistic study and organoleptic characters was done by the VHCA Herbals, Ayurveda LLP, Gharaunda.

**CHEMICAL AND REAGENTS**

Scopolamine was purchased from S.D. Fine Chemicals, Mumbai. Piracetam (Ceracetam Tablet 800mg) used as Standard drug.

**METHODS:**

**Preparation Of Ethanolic Extract**

The powder of plant leaves was subjected to soxhlet extraction ethanol as a solvent system for 72hr. The extract was filtered and concentrated in vaccum under reduced pressure using rota rod flash evaporator. Allowing complete evaporation of solvent on a water bath and then finally vaccum dried. The yield of ethanolic crude extract for 80gm of powder was 6.5gm.

**Calculation of percentage yield**

The percentage yield extract was calculated by using following formula:

Percentage yield = Weight of extract/ Weight of powdered drug taken * 100.

**Determination of Acute toxicity and $LD_{50}$:**

The acute toxicity of prepared extract was performed using OECD guideline 425 in a following manner:

**EXPERIMENTAL ANIMALS**

An experiment was performed in accordance with the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) guidelines after the approval of the experimental protocol by the Institutional Animals Ethical Committee (IAEC).

The Wistar rats of 12-16 weeks were be used for the study. The animals were housed (5-6) per cage at temperature (25±1°C) with 50±55% of relative humidity under 12 h day and night cycle and fed standard rodent chow and water add libitum.

**A. Selection of animal species and housing**:

All the test animal were kept in separate cages at least 5 days before the commencement of toxicity test. Animals were maintained at 22±3°C in (12:12) light and dark cycle with free access to food and water.

**B. Preparation of doses:**

During the study fresh aqueous solution of extract of Artemisia absintium was made and each dose was administered by varying the concentration of the drug extract.

**C. Test procedure**

The required dose is administered in animal one at a time by using oral gavage The animals (Rats) were fasted overnight but water was not withdrawn. The fasted body weight of rat is determined and dose is calculated on body weight basis. After administration of Artemisia absinthium extract the food is withheld for further 3-4 h. For limit test 2000 mg/kg dose was administered in one animal and then the animal was observed for mortality for a period of 48hr,if the tested rat survived, the test was continued by taking 4 more animals.

In main test, dose of 1.75, 5.5, 17.5, 55, 175, 550, 2000 was selected and was administered in animal one at a time. The animal was observed for any toxic symptoms initially for 1hr. interval for 4 hrs, then periodically for up-to 14 days.

**D. Selection of Dose groups:**

**1.** On the basis of acute toxicity study data. It was conclude that LD50 of Artemisia absintium extract is safe upto 2000mg/kg.

**2.** Therefore the test groups were divided as 100mg/kg (low dose),200 mg/kg (medium dose), 400mg/kg (high dose).

**TREATMENT PROTOCOL:**

**Groups of animals :** In each model the animals is divided into 6 group,6 animals each n=6

1) Control group 10ml/kg (p.o)
2) Scopolamine (5mg/kg i.p.)
3) Standard group (piracetam 600mg/kgi.p.) and scopolamine (5mg/kgi.p.)
4) EEAA (100mg/kg) and scopolamine (5mg/kgi.p.)
5) EEAA (200mg/kg) and scopolamine (5mg/kgi.p.)
6) EEAA (400mg/kg) and scopolamine (5mg/kgi.p.)

**EVALUATION OF ANTIAMNESIC ACTIVITY:**

**Evaluation Parameter**

1} Determination of Step down latency (Passive avoidance test)
2} Determination of escape latency (Morris water maze) :
3} Discrimination index (object recognition test)

**Object Recognition Model**

Object recognition apparatus consist open white colored plywood box (70×60×30 cm.) with a well furnished floor. The box is illuminated by 60 w lamp suspended above the box. The object to be discriminated made of plywood in two different shape of 8 cm and colored black and white. The object recognition test is a behavioral test that is widely used to examine animal's memory performance. Memory performance in the ORT is based on the natural tendency of animals to explore novel objects. The day before the test, rat was given habituation session where they were left to freely exploring the box for 2 min. No object was placed in the box during the habituation trial. On the day of test, two identical objects were presented in two opposite corner of the box during the first trial(T1.). and the amount of time taken by each rat to complete 20 s of object and/or touching it with nose or forepaw. Turning around or sitting on the object was not considered as an exploratory behavior. During the second trial (T2, 90 min. after T1 ) one of the objects presented in the T1 (i.e. familiar objects) was replaced by new object and rat was left in box for 5 min. The time spent (s) for exploration of the familiar (F) and new (N) object was recorded and discrimination index was calculated.

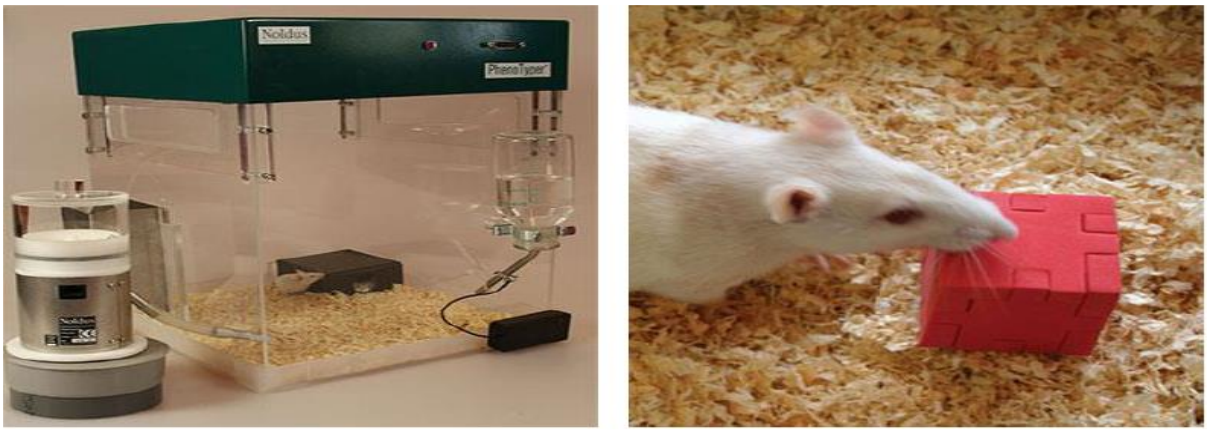



**Figure 01 : Object Recognition Test Apparatus.**

Discrimination Index = N-F/ N+F
Where, N= Exploration of the new object
F= Exploration of the familiar object
Scopolamine (5mg/kg) was injected i.p. after 45 min. of administration of Artemisia absinthium (100,200,400 mg/kg) or piracetam (600mg/kg)or vehicle in Rat and trial was given 45 min after injection of scopolamine .

**Passive Avoidance Test**

Passive avoidance test apparatus consist of (27×27×27) having three walls of wood and one wall of plexiglass feauturing a grid floor (3 mm. of stainless steel rods set 8 mm apart) with a wooden platform (10×7×1.7) in the center of grid floor. The box is illuminated with 15 w during the experimental period. Electric shock (20 v AC) was delivered to the grid floor. Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. Training was carried out in two similar sessions. Each rat gently placed on the wooden platform set in the center of the grid floor. When the rat stepped down and placed all its paw's on the grid floor.Shock was delivered for 15 s and step down latency was recorded. SDL was defined as the time taken by the rat to step down from wood platform to grid floor with with all its paws. Animals showing in the range (2-15 s) during the test were used for the second session and the retention test. The second session was carried out after 90 min. of first test. When the animals stepped down before 60 s. electric shock was delivered for 15 sec. During the second test, the animals removed from the shock free zone if they did not step down for 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each rat again placed on the platform and the SDL was recorded with upper cut off time 300s.

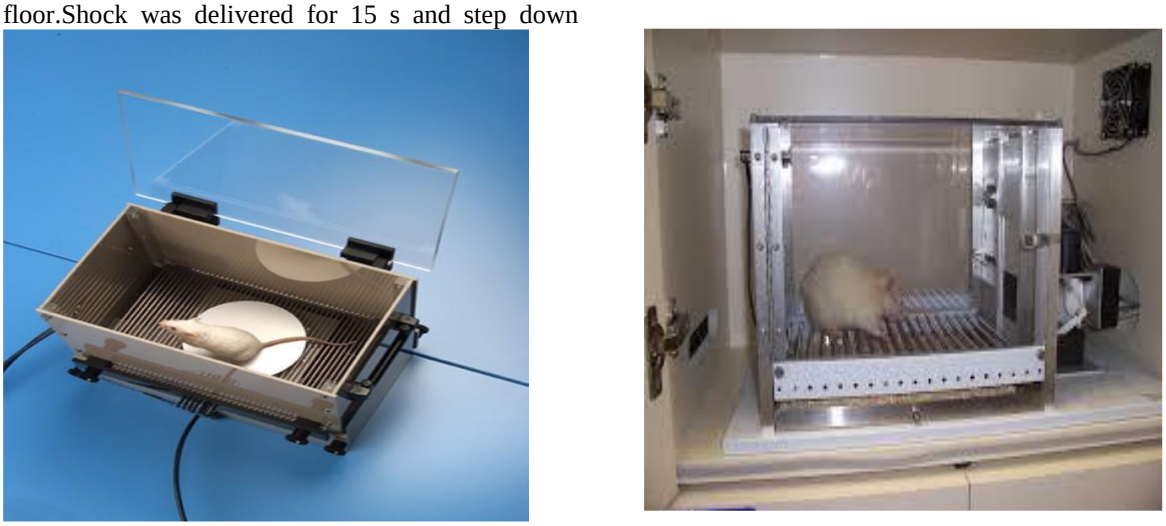


**Figure 02: Passive Avoidance Test Apparatus.**

Artemisia absintium extract (100,200,400 mg/kg p.o.), piracetam (600 mg/kg i.p.) or vehicle were administered orally for 8 days and SDL was recorded after 45 min. of administration of last dose on eight day and again after 24 h i.e. on ninth day, in the scopolamine treated group,
Scopolamine(5mg/kg) was injected i.p. after 45 min of administration of extract or piracetam or vehicle and SDL was recorded after of injection of scopolamine on eighth day and after 24 h. i.e.on ninth day.

**Morris Water maze**

The Morris water maze is a circular pool (100 cm. in diameter and 45 cm. in height) with a featureless inner surface. The circular pool was filled with a water in which 500ml. of milk had been mixed to a height of 30 cm. (20±1 c). The pool was divided into four quadrants of equal area. A white platform (6 cm. in diameter and 29 cm. in height) was centered in one of the four quadrants of the pool and submerged 1 cm below the water surface so it was invisible at water level. The spatial memory test was performed by the method of Morris .in the water maze experiments, the day prior to experiment was dedicated to swim training for 60 s in the absence of the platform, in the days following the mice were given two trial sessions each day for consecutive days, during each trial, the escape latencies of rat were recorded . This parameter was averaged for each session of trials and for each rat. Once the mouse located the platform, it was permitted to remain on it for 10s. if the mouse did not locate the platform within 120s, it was placed on the platform for 10s. and then removed from the pool by experimenter. The rat was given two daily trials for four consecutive days with an inter-trial interval of 20 min. The of entry of rat in to the pool and location of the platform for escape remained unchanged between trials 1 and 2 but was changed each day thereafter. The in escape latency from day to day in trial 1 represents long term memory or reference memory while that from trial 1 to trial 2 represents short term memory or working memory.

**Figure 03: Morris Water Maze Test Apparatus.**

Rats were treated with water for injection and Artemisia absinthium extract preparation before the training trial. After 90 min, amnesia was induced mice with scopolamine given intraperitoneally

**Statistical Analysis**

Data were expressed as the mean ± standard error of mean (SEM) and statistical analysis were carried out employing one way analysis of variance (ANOVA) followed by Dunnett test.

## 3. RESULTS:

**Percentage Yield Of Extract:**

Weight of Artemisia Absinthium: 80 gm
Wt. of extract obtained: 6.5gm
The yield of Artemisia absinthim extract was 6.5 gm for 80gm.
Therefore,

$$\% \text{ practical yield} = \frac{\text{practical yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{6.5}{80} \times 100$$

$$= 8.125 \text{ \% w/w}$$

The % practical yield of ethanolic extract of Artemisia absinthium was found to be 8.125 % w/w.

**Qualitative phytochemical screening**

**Table no. 01: Qualitative phytochemical screening of Ethanolic extract of Artemisia absinthium-**

| Sr. no | Phytochemical Screening | Ethanolic Extract | |
|---|---|---|---|
| | | Observation | Inference |
| 1. | Flavonoids | Flavonoids dissolve giving red color | Presence of flavonoids |
| 2. | Steroids | Greenish color | Presence of steroids |
| 3. | Phenols | Blue or Dark green | Presence of phenols |
| 4. | Saponins | Copious lather formation | Presence of saponins |
| 5. | Alkaloids | Yellow color | Presence of alkaloids |
| 6. | Tannins | Dark blue or black color | Presence of tannins |
| 7. | Proteins | Violet color | Presence of proteins |
| 8. | Quinones | Red color | Presence of quinones |

**Pharmacological Study:**

**Acute Toxicity**

**Table No. 02. Observation Of Acute Toxicity Study**

| Sr No. | Dose (Mg/Kg) | Observation |
|---|---|---|
| 1 | 5.5 | No Death |
| 2 | 17.5 | No Death |
| 3 | 55 | No Death |
| 4 | 175 | No Death |
| 5 | 550 | No Death |
| 6 | 2000 | No Death |

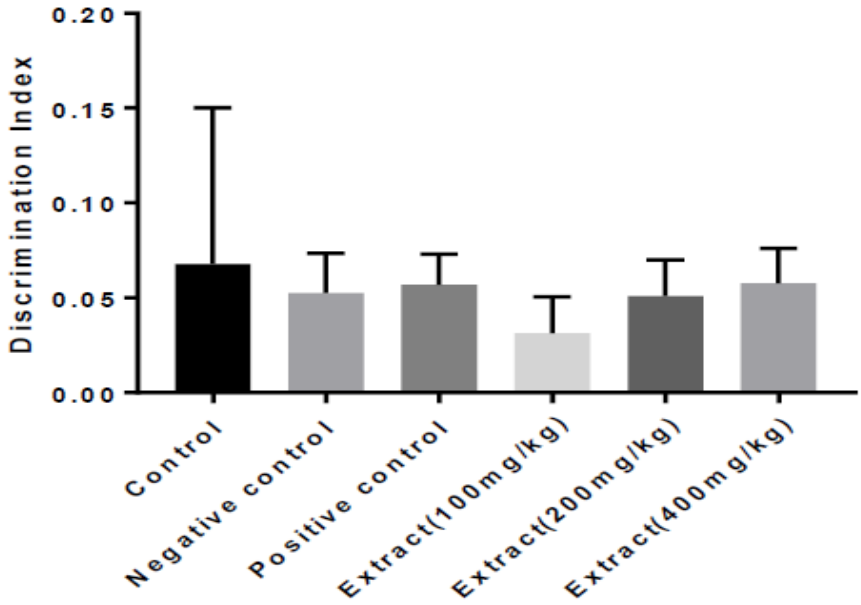
Acute Toxicity Studies (OECD – 425 Guidline) Of Artemisia Absinthium Revealed That The Extract Was Safe Upto 550 Mg/Kg Dose And The Dose Above 550 Produce Mild Degree Of Sedation And Imbalance In Locomotor Activity But, No Death Observed Up To The Dose 2000 Mg/Kg. Therefore The Ld50 Of Ethanolic Extract Of Artemisia Absinthium Was Found To Upto 2000mg/Kg.

**Object Recognition Test:**

**Table 03. Effect Of EEAA On Discrimination Index By Object Recognition Model.**

| Group | Treatment And Doses | Discirmination Index |
|---|---|---|
| **1.** | Control 10ml/Kg (P.O) | 0.06780±0.03361** |
| **2.** | Negative Control Scopolamine (5mg/Kg I.P.) | 0.05233±0.008620*** |
| **3.** | Positive Control (Piracetam600mg/Kg,I.P) +Scopolamine (5mg/Kg I.P) | 0.05692±0.006558 |
| **4.** | EEAA.(100mg/Kg P.O)+ Scopolamine(5mg/Kg I.P.) | 0.03140±0.007744** |
| **5.** | EEAA.(200mg/Kg P.O)+ Scopolamine (5mg/Kg I.P.) | 0.05092±0.007852** |
| **6.** | EEAA.(400mg/Kg P.O)+ Scopolamine(5mg/Kg I.P) | 0.05450±0.005356** |

Results Are Expressed As Mean ± Sem (N=6). Data Was Analyzed By One Way Analysis Of One Way-(Anova) Followed By Dunnett Test. ***P*<0.001 When Compared With Control Groups And ***P>0.001when Compare With Negative Control Group.




**Graph 01: Effect of Eeaa on Object Recognition Test In Scopolamine Induced Amnesia In Rat.**

From The Table 7.3 & Graph 7.1, Results Indicates That The Discrimination Index Of Eeaa (100,200,400mg/Kg) Was Found To Be Increased As Compare To Control Group In A Dose Dependent Manner.

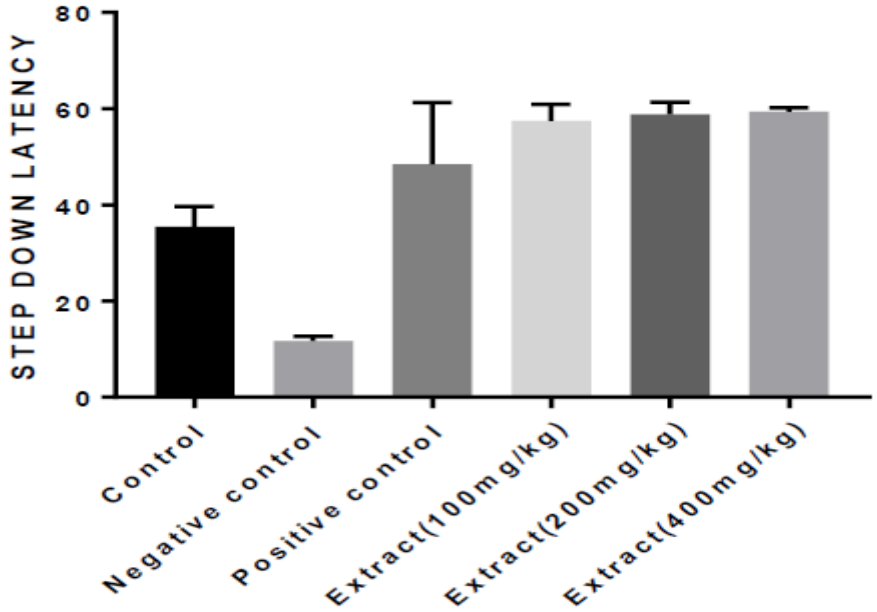
**Passive Avoidance Test**

**Table 04. Effect Of EEAA On Step Down Latency (Sdl) By Passive Avoidance Test.**

| Group | Treatment&Doses | Mean & A.A(Step Down Latency) | |
|---|---|---|---|
| | | Acquisition Trial 8th Day | Retention Trial 9th Day |
| I | Control(10ml/Kg P.O.) | 35.500±1.727*** | 75.167±1.447*** |
| II | Negative Control Scopolamine (5mg/Kg I.P.) | 11.667±0.4216*** | 26.833±0.6540*** |
| III | Positive Control (600mg/Kg I.P.) +Scopolamine (5mg/Kg I.P.) | 59.000±3.215 | 175.00±1.826 |
| IV | EEAA(100mg/Kg P.O)+Scopolamine(5mg/Kg I.P ) | 57.500±1.384*** | 168.17±0.7032*** |
| V | EEAA(200mg/Kg P.O)+ Scopoalmine (5mg/Kg I.P) | 58.833±1.046*** | 172.17±1.4933*** |
| VI | EEAA(400mg/Kg P.O.)+Scopolamine (5mg/K I.P.) | 59.333±0.333*** | 174.17±1.579*** |

Results Are Expressed As Mean ± Sem (N=6). Data Was Analyzed By One Way Analysis Of Variance (Anova) Followed By Dunnett Test. **** *P*<0.001 When Compared With Control Groups And ***P<0.001 When Compare With Negative Control Group.

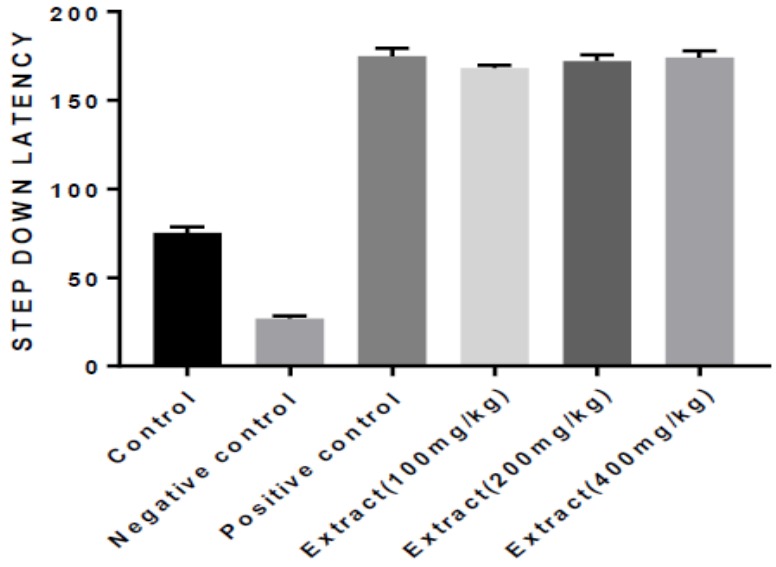
**A] Acquisition Trial (60sec) For 8th Day**




**Graph 02. Effect Of EEAA On Passive Avoidance Test Using A Scopolamine-Induced Amnesia NModel In Rats On 8th Day** .

From Table 7.4 & Graph 7.2, Result Shows That The Step Down Latency On 8th Day Of Eeaa (100,200,400mg/Kg) Was Found To Be Increased As Compare To Control Group In Dose Dependent Manner.

**B] Retention Trial (180 Sec.) For 9th Day**




**Graph 7.3 Effect Of** EEAA **On Passive Avoidance Test By Using A Scopolamine-Induced Amnesia Model In Rats On 9th Day.**

From Table 7.4 & Graph 7.3, Shows A Result That The Step Down Latency On 9th Day Of **EEAA** (100,200,400mg/Kg) Was Found To Be Increased As Compare To Control Group In Dose Dependent Manner.

**Morris Water Maze**

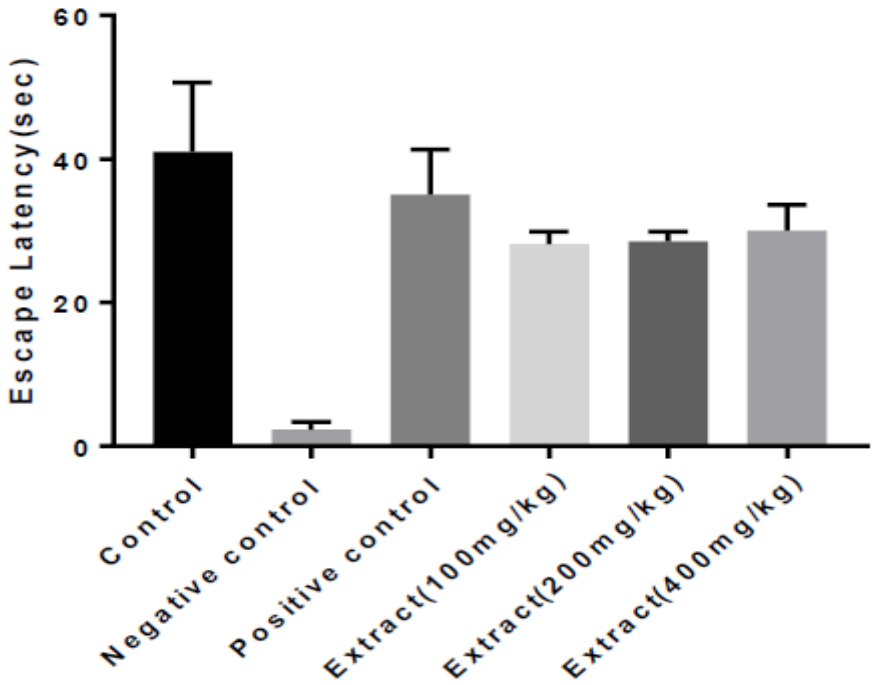
**Table 05. 4 Acquisition Trials Per Day For 4 Consecutive Days**

| Groups | Treatment & Doses | 1st Day | 2nd Day | 3rd Day | 4th Day |
|---|---|---|---|---|---|
| **GROUP I** | Control 10ml/Kg(P.O) | 41.16±1.249 | 36.16±0.9458 | 31.50±0.7638 | 24.83±1.447 |
| **GROUP II** | Negative Control Scopolamine (5mg/Kg/I.P) | 116.17±2.358 | 114.67±2.667 | 113.67±2.728 | 112.67±2.704 |
| **GROUP III** | Positive Control Piracetam(600mg/Kg)(I.P)+ Scopolamine(5mg/Kg)(I.P.) | 55±1.000 | 55.83±0.6009 | 52.667±0.6667 | 50.833±2.414 |
| **GROUP IV** | EEAA (100mg/Kg/P.O.)+ Scopolamine(5mg/Kg)( I.P.) | 53.50±0.9916 | 52.667±0.8819 | 51.667±0.5578 | 50.83±2.272 |
| **GROUP V** | EEAA (200mg/Kg)(P.O)+ Scopolamine(5mg/Kg) (I.P.) | 55.16±0.8724 | 54.83±1.014 | 52.83±0.7491 | 51.66±1.926 |
| **GROUP VI** | EEAA (400mg/Kg/P.O)+ Scopolamine(5mg/Kg)( I.P.) | 56.33±0.8763 | 54.66±1.0145 | 54±0.7492 | 53.16±1.936 |

**Table 06. Probe Trial (Retention) Of The Morris Water Maze Tasks For 5th Day:**

| Groups | Treatment & Dose | Probe Trial (5th Day) |
|---|---|---|
| Group I | Control 10ml/Kg (P.O) | 41.00±3.950**** |
| Group II | Negative Control Scopolamine (5mg/Kg)( I.P) | 2.333 ±0.4216*** |
| Group III | Positive Control Piracetam (600mg/Kg I.P)+Scopolamine(5mg/Kg I.P.) | 35.000±2.582 |
| Group IV | EEAA(100mg/Kg/P.O)+ Scopolamine(5mg/Kg)( I.P.) | 28.167±0.7032**** |
| Group V | EEAA(200mg/Kg/P.O)+ Scopolamine(5mg/Kg)(I.P.) | 28.500±0.5627**** |
| Group VI | EEAA(400mg/Kg/P.O)+ Scopolamine(5mg/Kg)( I.P.) | 30.00±1.461**** |

Results Are Expressed As Mean ± Sem (N=6). Data Was Analyzed By One Way Analysis Of Variance (Anova) Followed By Dunnett Test. **** *P*<0.001 When Compared With Control Groups And ***P<0.001 When Compare With Negative Control .




**Graph 04: Effect Of EEAA On Probe Morris Water Maze Task By Using Scopolamine Induced Amnesia In Rat.**

From Table 7.6 & Graph 7.4, Results Shows That The Escape Latency Of Eeaa (100,200,400mg/Kg) Was Increased On 5th Day As Compare To Control Group In Dose Dependent Manner.

## 04. DISCUSSION:

Free radicals are chemical species possessing an unpaired electron. The radical derivatives of oxygen (O2) are the most important free radicals in the biological systems. These radicals are involves in the pathogenesis of many inflammatory diseases like Alzheimer's disease.

The clinical features of Alzheimer's disease (AD) are coupled with a progressive loss of neurons in several different regions of the brain. One theory on the pathogenesis of AD postulates that neurodegeneration is the result of oxidative stress and damage to vulnerable cerebral tissues. Scientists have known for some time that certain proteins accumulate in the brains of Alzheimers patients, leading to nerve cell damage. Exactly what causes the toxic plaques to form has not been established, antioxidants have the ability to bind and inactivate these destructive radicals, can combat the plaques. Under normal circumstances, the brain is protected from such damage by a careful balance between pro-oxidant and antioxidant mechanisms which include antioxidant enzymes A.

In the present study, extract of Artemisia absinthium (100,200,400mg/kg) improved learning and memory of rat significantly in interoceptive behavioral models employed. The simultaneous analysis or a distinction between reference and working memory is well established through ORT, MWM and PAT. Scopolamine a nonselective muscarinic antagonist blocks cholinergic signaling and produce memory deficit that are similar to those found in age related senile CNS dysfunction. The enhancement of memory in scopolamine induced animal model is also associated with altered status of brain oxidative stress. Scopolamine interferes with memory and cognitive function and subsequently causes enhancement of reference (long term) and working (short term) memories. Rat were given scopolamine to induce memory enhancement at a dose of 5mg/kg.

In the present study, the effect of Artemisia absinthium (100,200,400mg/kg) was examined on the performance of rat in an object recognition task that has been considered to be pure working memory task. Rat are able to discriminate between a familiar object and new object 1hr. or less, but not 24 h. after the presentation of familiar task. The effect of Extract of Artemisia absinthium (100,200,400mg/kg) was investigated on the acquisition of the information and on the consolidation of memory that takes place shortly after the acquisition and on the restitution of the information. The result indicated that the rat spend

more time in exploring a new object than a familiar object in the scopolamine treated grp. When pretreated with extract of Artemisia absinthium. The Discrimination index decreased in the scopolamine treated group. Pretreatment with extract of Artemisia absinthium significantly increased the DI when compared with respective control. 200mg/kg and 400mg/kg of EEAA was found to be effective to enhance memory task.

Thus result demonstrate that improved retention in rat subjected to object recognition task in the scopolamine improves the consolidation and possibly the acquisition phase of working memory that is altered in interoceptive memory deficit models, i.e. scopolamine treated group. Piracetam(600mg/kg) established nootropic agents used as a standard in the present study also significantly improved the DI.

The ameliorative effects of EEAA on learning and memory were investigated in the passive avoidance task. Scopolamine treated rat significantly shorter step down latencies. EEAA (100,200,400mg/kg) treatment showed a significant increase in SDL in rat. Pretreatment with EEAA significantly decreased SDL in the scopolamine treated group. Piracetam used as the positive control also increased the step down latency. 100,200,400mg/kg dose of Artemisia Absinthium was found to be effective to reverse the amnesia induced scopolamine.

The simultaneous analysis for a distinction between reference and working memory is well established through the Morris water maze test. In our experiments, normal control rat exhibited well formed reference memory and working memory. By contrast, rat given scopolamine exhibited neither reference nor working memory. EEAA(100,200,400mg/kg) improved the amnesic deficits in reference memory and working memory. The prolongation in escape latency induced by scopolamine was significantly and gradually decreased over the four testing days. Mean escape latency and EEAA on first day and on fourth day, 51.83,49.83, and 47.16 of dose on successive days.

The above behavioural result suggests that EEAA has the ability to improve and ameliorate spatial long and working memory.

Many clinical studies have reported strong evidence that oxidative stress is involved in the pathogenesis of AD. Oxidative stress refers to the undue oxidation of biomolecules leading to cellular damage, and it occcurs by reactive oxygen species. Amounts of oxidatively modified proteins, lipids and DNA; such free radicals-mediated molecular damage is particularly prominent in the environment of senile plaques and in neurofibrillary tangle bearing neurons, suggesting roles for ROS in amyloid mediated neuronal damage and neurofibrillary pathologies. Several sources of ROS in AD have been proposed, with amyloid beta protein and redox metals such as $Fe_2$ or $Cu_2$ being two such sources.

The drugs with antioxidant effects might be beneficial for processing brain function. Antioxidant enzymes such as superoxide dismute (SOD), glutathione peroxidase and catalase as well as glutathione reductase and ascorbate are involved in the reduction of oxidative stress. Antioxidant enzymes display the reduced activities in the affected brain region of patients of Alzheimer's disease, morever the reduction in the level of intracellular oxidized protein under these conditions has been in the level of intracellular oxidized protein under these conditions has been associated with the improvement of cognitive and or psychomotor functions. These agent reduce the oxidative damage and promote a functional recovery in neurodegeneration disorder. In the course of searching natural products with memory enhancing activity using scopolamine induced amnesic mouse as an experimental model for AD. It was found that extract of Artemisia absinthium showed a significant memory enhancing activity in ORT, MWM and PAT.

The observed beneficial effects of extract of Artemisia absinthium may be attributed to its diversified chemical components namely flavonoids, Alkaloids, and tannins. There are evidences which shows that flavonoids, alkaloids and tannins exhibit potential antioxidant property and free radical scavenging activity. Phytochemical screening of Artemisia plant may exhibit antioxidant property. Acetylcholine is is neurotransmitter involved in memory and cognitive function. There are also evidences found about whole plant that it showed a anticholinestrase activity. Superoxide dismutase and glutathione peroxidase are the endogenous antioxidant which decreases the oxidative stress. There is a evidences plant may exhibit a neuroprotective action and may help in the release of endogenous antioxidant.

## 05. CONCLUSION:

From the result of present investigation, it may be concluded that leaves of Artemisia absinthium enhances the cognitive activity of brain. It may be improve the short term memory and long term memory. Antioxidant, anticholinsterase and neuroprotective role may be responsible for a cognitive enhancing effect. Hence, Artemisia absinthium may be useful in the treatment or prevention of various cognitive disorders.

From Ethanomedicinal studies on Artemisia absinthium, define that the future aspect about Artemisia absinthium have revealed its pharmacological potential, which is essential for its further consideration andstandardization as a medicine at safer level. Future Studies are required to isolate the constituent and its modulation with Agonist & Antagonist to find out the exact mechanism of neurotransmitters involved.

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**07. COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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