To Study the Cognitive Enhancing Activity of Artemisia Absinthium on Scopolamine Induced Amnesia In Rat.

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Abstract: 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). Scopolamine is used as a standard/reference drug for inducing amnesia in man and animals. Recent lesion studies suggest that the role of acetylcholine may not be specific to memory functions but point towards involvement in attention and in some forms of synaptic plasticity. 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 ad libitum.

Keywords: Artimisia Absinthium, Scopolamine, Wister Rats, Soxhlet extraction.

INTRODUCTION

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 publically. 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. The brain of patient with alzheimer’s dramatically shrinks over the uration of the disease. Key areas of the brain such as the hippocampus and cerebral cortex will become smaller as massive amounts of neurons
die. The brain of an alzheimer’s disease patient will appear much tinier than the brain of a normal aged adult. Larger gaps will be present in areas that should otherwise have grey and white matter. Alzheimer’s disease mostly targets the cerebral cortex, resulting in degeneration of the frontal cortex, cingulate gyrus, temporal lobe, and parietal lobe. This explain a number of alzheimer’s disease symptoms including speech and language difficulties, concentration and cognitive problems, and disorientation. It is important to note that neurons in the hippocampus will die. The hippocampus is the main area of the brain responsible for memory function, which is primary reason why alzheimer’s patient having several memory impairment.

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. Groups of nerve cells in our brain have special jobs. Some are involved in thinking, learning and memory. Others help us see, hear, smell and tell our muscles when to move. Brain cells operate like tiny factories. They receive supplies, generate energy, construct equipment and get rid of waste. Cells also process and store information and communicate with other cells.

![Image of brain](image)

**Fig.1.1 Shrinkage of brain in Alzheimer disease**

### 1.1 Causes of Alzheimer Disease :

Alzheimer 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, Alzheimer’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.⁶
1.2 Clinical Features of Alzheimer Disease:

1.2.1 MILD ALZHEIMER 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.

1.2.2 MODERATE ALZHEIMER 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

1.2.3 SEVERE ALZHEIMER DISEASE –
- Inability to communicate
- Weight loss
- Seizures
- Skin infections
- Difficulty swallowing
- Groaning, moaning, or grunting (?)

2. 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.

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

3. EXPERIMENTAL ANIMALS
Experiments 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 ad libitum.

3.1 Methods:

3.1.1 Preparation of Ethanolic extract

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

3.1.2 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

3.1.3 Solubility analysis:

The solubility analysis of ethanolic extract of Artemisia absinthium has been carried out using different solvent.

3.1.4 Selection of Dose groups:

1. On the basis of acute toxicity study data. It was conclude that LD_{50} of Artemisia absinthium 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).

3.1.5 TREATMENT PROTOCOL:

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

1) Control group 10ml/kg (p.o)

2) Scopolamine (5mg/kg i.p.)

3) Standard group (piracetam 600mg/kg.i.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.)

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.

4. RESULTS:

4.1 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.
4.2 Qualitative phytochemical screening:
Table no. 1 Qualitative phytochemical screening of Ethanolic extract of Artemisia absinthium-

<table>
<thead>
<tr>
<th>SR .NO</th>
<th>PHYTOCHEMICAL SCREENING</th>
<th>ETHANOLIC EXTRACTS</th>
<th>OBSERVATION</th>
<th>INFERANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoids</td>
<td>Flavonoids dissolve giving Red colour</td>
<td>Presence of flavonoids</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Steroids</td>
<td>Greenish color</td>
<td>Presence of steroids</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Phenols</td>
<td>Blue or Dark green</td>
<td>Presence of phenols</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>Copious lather formation</td>
<td>Presence of saponins</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloids</td>
<td>Yellow color</td>
<td>Presence of alkaloids</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Tannins</td>
<td>Dark blue or black color</td>
<td>Presence of tannins</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Proteins</td>
<td>Violet color</td>
<td>Presence of proteins</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Quinones</td>
<td>Red color</td>
<td>Presence of quinones</td>
<td></td>
</tr>
</tbody>
</table>

4.3 PHARMACOLOGICAL STUDY:

Acute Toxicity
Table no. 2 Observation of Acute toxicity study

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>DOSE MG/KG</th>
<th>OBSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.5</td>
<td>No Death</td>
</tr>
<tr>
<td>2.</td>
<td>17.5</td>
<td>No Death</td>
</tr>
<tr>
<td>3.</td>
<td>55</td>
<td>No Death</td>
</tr>
<tr>
<td>4.</td>
<td>175</td>
<td>No Death</td>
</tr>
<tr>
<td>5.</td>
<td>550</td>
<td>No Death</td>
</tr>
<tr>
<td>6.</td>
<td>2000</td>
<td>No Death</td>
</tr>
</tbody>
</table>

Acute toxicity studies (OECD – 425 guideline) 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 2000 mg/kg.
Object Recognition Test:

Table 3  Effect of EEAA on Discrimination index by object recognition model.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT &amp; DOSES</th>
<th>DISCRIMINATION INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control 10ml/kg (p.o)</td>
<td>0.0678±0.0336**</td>
</tr>
<tr>
<td>2.</td>
<td>Negative Control Scopolamine(5mg/kg i.p.)</td>
<td>0.0523±0.008620***</td>
</tr>
<tr>
<td>3.</td>
<td>Positive Control (Piracetam 600mg/kg,i.p)</td>
<td>0.0569±0.006558</td>
</tr>
<tr>
<td>4.</td>
<td>EEAA.(100mg/kg p.o)+ Scopolamine(5mg/kg i.p.)</td>
<td>0.0314±0.007744**</td>
</tr>
<tr>
<td>5.</td>
<td>EEAA.(200mg/kg p.o)+ Scopolamine(5mg/kg i.p.)</td>
<td>0.0509±0.007852**</td>
</tr>
<tr>
<td>6.</td>
<td>EEAA.(400mg/kg p.o)+ Scopolamine(5mg/kg i.p.)</td>
<td>0.0545±0.005356**</td>
</tr>
</tbody>
</table>

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.
Recognition Test in Scopolamine induced Amnesia in Rat.

From the Table 1 & Graph 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.

Table 7.6  Probe Trial (Retention) of the Morris water maze tasks for 5th day:

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.

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 and standardization as a medicine at safer level. Futhure Studies are required to isolate the constituent and its modulation with Agonist & Antagonist to find out the exact mechanism of neurotransmitters involved.

References:


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