EVALUATION OF NOOTROPIC ACTIVITY OF ACOROUS CALAMUS AGAINST SCOPOLAMINE INDUCED ALZHEIMER'S

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ABSTRACT

Alzheimer's disease is an age-associated, irreversible, progressive neurodegenerative disease that is characterized by severe memory loss, unusual behavior, personality changes and a decline in memory function. Several scientific studies have described the use of various medicinal plants and their constituents for the treatment of Alzheimer's disease. Acorous calamus [sweet flag] is a well known plant which is being used in Indian traditional medicine, as anti-oxidant of CNS associated disorders. Methods: In the present study, albino Wistar rats were used and divided in to 5 groups each group consists 6 animals. Group I treated as control group (Normal saline), Group II treated as negative control (Scopolamine 1 mg/kg i.p), Group III and IV treated as experimental low and high doses of plant extract (200, 400 mg/kg p.o), Group V treated as standard group (piracetam 150mg/kg i.p) respectively. Elevated plus maze, Y-maze, Morries water maze task are the behavioral models for the testing of Alzheimer's disease. Scopolamine induced Alzheimer's served as the interoceptive behavioural models. Results: The results suggested that dried rhizome ethonolic extract of Acorous calamus has potential effects against to the scopolamine induced Alzheimer's by regulating AchE activity, free radical scavenging activity. Conclusion: It is concluded that the dried rhizome ehonolic extract of Acorous calamus having the ability to reduce the progenosis of Alzheimer's disease in rats.

KEYWORDS: Acorous calamus, Alzheimer's, Scopolamine, Anti-oxidant, AchE.

INTRODUCTION

Alzheimer's disease is an age-associated, irreversible, progressive neurodegenerative disease that is characterized by severe memory loss, unusual behaviour, personality changes and a
decline in memory function. It is the most common form of dementia and affects an estimated 10 million people worldwide. Alzheimer's disease demolishes the vital brain cells, causing trouble with memory, thinking and behaviour, brutal enough to affect work, lifelong hobbies and social life. Recognized factors in Alzheimer's disease include acetylcholine deficiency, free radicals and inflammation of the brain tissue. There is no cure for Alzheimer's disease, but drugs designed to slow down the disease progression are available. Some herbs may help to improve brain function, but scientific evidence to prove that they can treat Alzheimer's disease. Medicinal plants have been the single most productive source of leads for the development of drugs, and over a hundred new products are already in clinical development. Indeed, several scientific studies have described the use of various medicinal plants and their constituents for treatment of Alzheimer's disease (Mohamed Abdel Galil Hassan, 2014).

This condition is characterized by a progressive loss of memory, deterioration of virtually all intellectual functions, increased apathy, decreased speech function, disorientation, and gait irregularities. Although the aetiology is unknown, genetic factors clearly play a role in 10% to 15% of cases. *Acorus calamus* (Sweet flag) has a very long history of medicinal use in Chinese and Indian herbal traditions. It is widely employed in modern herbal medicine as its sedative, laxative, diuretic, and carminative properties. It is used in Ayurveda to counter the side effects of all hallucinogens. *Acorus calamus* have shown antioxidant, antimicrobial and insecticidal activities. *Acorus Calamus* was also known to many early American settlers and used for a number of diseases (Pradeep et al., 2002).

**Pathology**
The brain has 100 billion nerve cells (neurons). Each nerve cell connects with many others to form communication networks. Groups of nerve cells have special jobs. Some are involved in thinking, learning and remembering. Others help us see, hear and smell. To do their work, 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. Keeping everything running requires coordination as well as large amounts of fuel and oxygen. The beta-amyloidal peptide (BAP), with 39 - 42 amino acid residues plays a significant role in the development of AD. Although there is no cure for AD, it can be managed with the available drugs, to some degree. Several studies have revealed that natural antioxidants, such as vitamin E, vitamin C and beta-carotene, may help in scavenging free radicals generated during the initiation and progression of this disease. The loss of memory is
considered to be the result of a shortage of the nerve transmitter acetylcholine. It is possible to increase the level of this transmitter in the brain by inhibiting the activity of the enzyme acetyl cholinesterase, which splits or breaks down the transmitter substance. Drugs that inhibit the breakdown of the messenger or transmitter acetylcholine delay the development of the disease (Kastenholz, 2011).

The brains of individuals with Alzheimer’s have an abundance of plaques and tangles. Plaques are deposits of a protein fragment called beta-amyloid that build up in the spaces between nerve cells. Tangles are twisted fibres' of another protein called tau that build up inside cells. Though autopsy studies show that most people develop some plaques and tangles as they age, those with Alzheimer’s tend to develop far more. They also tend to develop them in a predictable pattern, beginning in the areas important for memory before spreading to other regions. Scientists do not know exactly what role plaques and tangles play in Alzheimer’s disease. Most experts believe that they somehow play a critical role in blocking communication among nerve cells and disrupting processes the cells need to survive. The destruction and death of nerve cells causes memory failure, personality changes, problems in carrying out daily activities and other symptoms of Alzheimer’s disease (Kastenholz, 2011).

**Warning signs**

1. Memory loss that disrupts daily life.
2. Difficulty completing familiar tasks at home, at work or at leisure.
3. Confusion with time or place.
4. Trouble understanding visual images and spatial relationships.
5. New problems with words in speaking or writing.
6. Misplacing things and losing the ability to retrace steps.
7. Decreased or poor judgement.
8. Changes mood and personality.

**Experimental Design**

Cognitive dysfunction was induced by administering scopolamine (1mg/kg) through i.p. route. Piracetam was used as standard and all animals were divided into five groups of each six animals.
MATERIALS AND METHODS

A. Water maze task
The Morris water maze consists of large circular tank made of black opaque PVC or hard board coated with fiber glass and resin and then surface painted white (1.8-2.0m in diameter and 0.4-0.6m height). The pool is filled with water (20-22°C) to a depth of 0.3-0.4m and rendered opaque by the addition of small quantity of milk or non-toxic white colour. The pool is fixed with filling and draining facilities and mounted on a frame so that the water is at waist level. The floor of circular tank is marked off in to four equal quadrants arbitrarily designed north, south, east or west. And escape platform is made of plexiglass with a 13 cm square platform attached to a 34 cm long clear plexiglass cylindrical pedestal (3cm. Diameter) mounted on a 1sq. m (5mm thick) plexiglass base. The top of the platform is covered with a coarse material that provides a good grip for the rat when climbing on a platform. For the hidden platform task, water is added to circular tank to a level 2cm above the top of the platform. Water maze represents a versatile tool in which a number of distinct tasks can be measured. The simplest measure of performance is the Latency to escape from the water on to the hidden platform (Melanie-Jayne et al., 2012).

The platform remains fixed in the position during the training session. Each animal is subjected to four consecutive trials for four days (from 15th to 18th day) during which they are allowed to escape on to the hidden platform and allowed to remain there for 20 sec. Escape latency time to locate the hidden platform in water maze is noted as an index of acquisition or learning. In case the animal is unable to locate the hidden platform within 120 sec, it is gently guided by hand to the platform and allowed to remain there for 20 sec. On the 19th day, 60 min after last dose, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval and measured.

B. Y-maze task
Y-maze task is used to measure the spatial working through the spontaneous alternation of behaviour. The maze is made of black painted wood. Each arm is 4cm long, 13 cm high, 3 cm
wide at the bottom, 1cm wide at the top, and converges at an equal angle. Each rat is placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Wistar rats tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the Wistar rats know which arm they have already visited. The series of arm entries, including possible returns into the same arm, are recorded visually. Alternation is defined as the number of successive entries into the three arms, on overlapping triplet sets. The percentage of alternation is calculated as the ratio of actual alternations, defined as the total number of arm entries minus two, and multiplied by 100 (Nunomura et al., 2000).

C. Elevated Plus Maze
The elevated plus maze consisted of two open arms (50x1cm) crossed with two closed arms (50x10x4cm). The arms were connected together with a central square (10x1cm). The apparatus was elevated to the height of 7cm in a dimly illuminated room. The maze was placed inside a light and sound attenuated room. Wistar rats were placed individually at the end of an open arm of elevated plus maze (EPM) facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms. Transfer Latency (TL) was recorded.

Transfer latency (TL) was taken as the time taken by rat to move into one of the covered arm with all its four legs was gently pushed into one of the two covered arms and the TL was assigned as 9sec. The mouse was allowed to explore the maze for 1sec and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day. On the 19th day, 9min after the treatment of last dose first trial is given and after 24 hr TL was noted for second time (i.e. on 20th day). The inflexion ratio was calculated by the formula.

\[ IR = \frac{(L_0 - L_1)}{L_0} \]

Where, \( L_0 \) is the initial transfer latency (TL) in Sec on first time,
\( L_1 \) is the transfer latency (TL) in Sec on 2nd time.

Decrease IR indicates the induction of amnesia, and increased IR indicates in improvement in cognition and memory impairments (Singhal et al., 2012).

RESULTS
A. Elevated Plus Maze
The Inflexion ratio (IR) of the Group II animals were significantly (P< 0.001) decreased in
comparison with the Group I (normal control) animals. EEAC (200 & 400 mg/kg) dose dependently increased IR in Group III Group IV significantly (p< 0.001) in comparable with Group II.

Table: 2 Effect of EEAC on Inflexion ratio in rats by using Elevated Plus Maze.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>Treatment</th>
<th>Inflexion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Control</td>
<td>0.624 ± 0.057</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Negative Control</td>
<td>0.084 ± 0.035&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>0.282 ± 0.016&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>0.298 ± 0.084&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>0.398 ± 0.032&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed (Mean ± SEM, n=6)

<sup>x</sup> p<0.001 when compared to normal control.

<sup>a</sup> p<0.001 when compared to negative control group.

Figure: 1 Effect of EEAC on Inflexion ratio in rats by using Elevated Plus Maze.

B. Effect of EEAC on Y-Maze
The alzheimer's induced group (Negative control) indicated decrease in the alternation of behaviour by the (P<0.01) in comparison with the control group I. The results presented by the treatment groups shows significance by (P<0.01 and P<0.01) increase in the alternation of behaviour in respect of 200mg/kg of EEAC and 400mg/kg of EEAC.
Table: 3 Effect of EEAC on % Alternations in rats by using Y – Maze

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>Treatment</th>
<th>Percentage alternation (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Control</td>
<td>56.62 ± 0.234</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Negative Control</td>
<td>25.23 ± 0.285&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>35.25 ± 0.294&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>39.28 ± 0.274&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>42.91 ± 0.242&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed (Mean ± SEM, n=6)

<sup>x</sup> p<0.001 when compared to normal control,

<sup>a</sup> p<0.001 when compared to negative control group.

C. Effect of EEAC on Water Maze

There is an increase in escape latency in Negative control group when compared with the control group (P<0.01) of the two groups of amnesia induced animals, both showed decreased time to escape on to the escape platform. The group treated with 200mg/kg EEAC group 400mg/kg treated showed the significance of (P<0.01 and P<0.01) respectively.
Table: 4 Effect of EEAC on Escape Latency in rats by using Morris Water Maze.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>Treatment</th>
<th>Escape Latency; (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Control</td>
<td>11.46 ± 0.242</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Negative Control</td>
<td>43.48 ± 0.228&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>24.04 ± 0.464&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>19.20 ± 0.182&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>13.68 ± 0.212&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed (Mean ± SEM, n=6)

<sup>x</sup> p<0.001 when compared to normal control

<sup>a</sup> p<0.001 when compared to negative control group.

Figure: 3 Effect of EEAC on Escape Latency in rats by using Morris Water Maze.

1. Effect of EEAC on AchE Activity

Injection of Scopolamine significantly (P<0.01) increased the AChE activity when compared with control group. In the treated group there was a significance (P<0.05) reduction in enzyme levels on both 200mg/kg and 400mg/kg of EEAC treated Wistar rats. Values are expressed as Mean ± SEM of animals.

Table: 5 Effect of EEAC on AcetylCholinesterase activity.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>Treatment</th>
<th>AcetylCholinesterase Level; (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Control</td>
<td>13.33±0.72</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Negative Control</td>
<td>19.21±0.12&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>18.24±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>14.95±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>11.08±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Results are expressed (Mean ± SEM, n=6)

x p<0.001 when compared to normal control

a p<0.001 when compared to negative control group.

2. Effect of EEAC on Super Oxide Dismutase

SOD levels in the brain significantly reduced (P<0.01) in A 25-35 induced group when compared to control group. Treatment with EEAC at 200 mg/kg and 400 mg/kg dose level showed significant increase (P<0.01 and P<0.01) respectively when compared with negative control group.

Table: 6 Effect of EEAC on Superoxide Dismutase (SOD) Level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Pictogram/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>9.083 ± 0.3005</td>
</tr>
<tr>
<td>II</td>
<td>Negative Control</td>
<td>5.667 ± 0.2472^x</td>
</tr>
<tr>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>6.717 ± 0.061^a</td>
</tr>
<tr>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>10.85 ± 0.068^a</td>
</tr>
<tr>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>11.27 ± 0.1764^a</td>
</tr>
</tbody>
</table>

Results are expressed (Mean ± SEM, n=6)

x p<0.001 when compared to normal control

a p<0.001 when compared to negative control group.
3. Effect of EEAC on Glutathione Peroxidase

The GPx in the dementia induced Wistar rats (group II) shown significant (P<0.01) reduction in the enzyme activity when compared with control group. The treatment with EEAC at 200mg/kg and 400mg/kg shown the significance (P<0.05 and P<0.01) respectively when compared with negative control group.

Table: 7 Effect of EEAC on Glutathione Peroxidase (GPX) Level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Units/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>38.6 ± 0.0817</td>
</tr>
<tr>
<td>II</td>
<td>Negative Control</td>
<td>28.77 ± 0.089x</td>
</tr>
<tr>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>29.83 ± 0.077a</td>
</tr>
<tr>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>32.40 ± 0.074a</td>
</tr>
<tr>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>32.87 ± 0.056a</td>
</tr>
</tbody>
</table>

Results are expressed (Mean ± SEM, n=6)

x p<0.001 when compared to normal control

a p<0.001 when compared to negative control group.
4. Effect of Eeac on Lipid Peroxidation

A significant decrease (P<0.05) in MDA level was observed in brains of rats treated with EEAC 400mg/kg when compared with control group animals. Standard treated animals showed significant decrease (P<0.01) MDA levels.

Statistical significance test for comparison were done by ANOVA, followed by Dunnet’s multiple comparison test. Comparisons were done between:

a) Group I vs Group
b) Group II vs Group III, IV, V

Table: 8 Effect of EEAC on Lipid peroxidation Level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lipid peroxidation (n mol MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.36±0.28</td>
</tr>
<tr>
<td>II</td>
<td>Negative Control</td>
<td>8.78 ± 0.09³</td>
</tr>
<tr>
<td>III</td>
<td>EEAC (200mg/kg)</td>
<td>3.7±0.18a</td>
</tr>
<tr>
<td>IV</td>
<td>EEAC (400mg/kg)</td>
<td>2.5±0.57a</td>
</tr>
<tr>
<td>V</td>
<td>Piracetam (150mg/kg)</td>
<td>1.8±0.36a</td>
</tr>
</tbody>
</table>
CONCLUSION

*Acorus calamus Linn* is a well known plant which is being used in Indian traditional medicine, as an antioxidant of CNS associated disorders, still there are some scientific evaluation to been made. Hence this research is emphasized to make the evident effect of the Rhizome of plant on memory disorder representing alzheimer’s disease.

The investigation was carried out on cognitive impairment with relevance of the hypothesis, on Scopolamine induced AChE oxidative stress signaling and impaired behavioural performance.

- The spatial learning in water maze task showed the significant memory retention indicated by the decrease in escape latency, improvement of percentage alternations in Y maze and inflexion ratio in elevated plus at both dose levels of dried rhizome of EEAC in 200 mg/kg and 400 mg/kg respectively.
- EEAC at 200mg/kg and 400mg/kg had shown the significant reduction in the elevated enzyme level of acetylcholine esterase which indicates the potential to increase cognitive function through the decreased degradation of acetyl choline.
- The oxidative stress involved by the administration of Scopolamine produced neurotoxicity indicated that decreased levels of super oxide dismutase, glutathione peroxidase. Treatment of dried rhizome of EEAC shows the protection of these antioxidant enzymes on both 200mg/kg and 400mg/kg dose level respectively due to the rejuvenating property of the extract.
In conclusion from the observation, the neuroprotective activity of the plant *Acorus calamus* on alzheimer’s type of dementia is due to the inhibiting activity against AChE, free radical scavenging activity and they are expected to be a pivot sense in neurotoxicity.

REFERENCES


