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EVALUATION OF THE NOOTROPIC EFFECT OF *ACORUS CALAMUS* IN EXPERIMENTAL ANIMALS

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ABSTRACT

Management of cognitive disorders like dementia and Alzeheimer's disease has been challenging since no potential drug is available with proved efficacy. Some nootropic drugs like piracetam, aniracetam and cholinesterase inhibitors such as donepezil have found to exhibit severe toxic effects in elderly. In the present study, we assessed the nootropic potential of hydroalcoholic extract of *Acorus Calamus* on various behavioural models. The young animals treated with hydroalcoholic extract showed dose dependent reduction in transfer latency. The hydroalcoholic extract of *Acorus calamus* rhizomes has reversed the amnesia induced by Scopolamine and Sodium nitrite. Many mechanisms can be expected for its activity owing to the presence of the large number of chemical constituents. The *Acorus calamus* has reversed the scopolamine and sodium nitrite induced amnesia. Primarily it may act by potentiating the cholinergic transmission by inhibition of acetylcholinesterase and increased in level of brain acetylcholine. Acorus calamus enhances the glucose utilization resulting in negating the effects of scopolamine on learning and memory impairment although a great deal of evidence supports that immunomodulators affect learning and memory. The exact mechanism that underlies this effect is yet to be determined as it possesses immunostimulant activity. It also possesses antioxidant, anti-inflammatory, antiepileptic properties which makes very difficult to judge the mechanism of action. All these mechanisms may act in conjunction, facilitating the acquisition and retention of learned activity. The hydroalcoholic extract of AC shows memory enhancing activity, which needs to be substantiated by more models. Further study is necessary to determine the actual mechanism of action and to find out the active component responsible for its activity.

INTRODUCTION:

Memory impairment is commonly seen by physicians in multiple disciplines including neurology, psychiatry, medicine and surgery[1]. Memory loss is often the most disabling feature of many disorders, impairing the normal daily activities of the patient and profoundly affecting their families. The key features of these dreaded disorders are memory impairments, deterioration of language, visuo-spatial, motor, sensory abnormalities, gait disturbances and seizures. There are around 30 million patients suffering from Alzheimer's disease (AD) which is the major cause of dementia, all over the world[2]. In India AD patients are estimated to be around 3 million[3]. Cognition is that operation of mind by means which, we become aware of our surroundings, objects and thoughts. Cognitive disorders such as delirium, dementia and amnesic disorders are common in elderly individuals. Memory

is vulnerable to a variety of pathologic processes including neurodegenerative diseases, strokes, tumors, head trauma, hypoxia, Cardiac surgery, malnutrition, attention deficit disorder, depression, anxiety, the side effects of medication and normal ageing[4]. Presently there are no satisfactory diagnostic procedures and therapeutic regimens available for the management of these cognitive disorders despite the severity and high prevalence of the diseases. Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, neurobiologists all over the world are looking for new directions and alternative strategies for managing cognitive disorders. The most common cause of dementia in elderly is probably Alzheimer's disease (AD), a chronic, progressive disabling organic brain disorder characterised by disturbance of multiple cortical functions, including memory, judgement orientation, comprehension, learning capacity and language[5]. Nootropic agents like piracetam and cholinesterase inhibitors like donepezil are commonly used for improving memory, mood and behaviour. However, the resulting adverse effects of these drugs such as diarrhoea, insomnia, nausea, bronchitis, loose stools, muscular cramps and other known side effects[6] have made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. The present studies deal with the evaluation of memory enhancement activity of hydroalcoholic extract of *Acorus Calamus*.

Materials and Methods

Chemicals used in the present study were scopolamine (Hi-Media laboratories Pvt. Ltd. Mumbai, India) piracetam, nootropil (UCB India Pvt. Ltd. Vapi, India), normal saline, hydroalcoholic extract of *Acorus Calamus*. Solution of scopolamine and piracetam was prepared in normal saline and injected intraperitoneally. All semi solid extract of *Acorus Calamus* were suspended in distilled water.

Animals Used: The healthy albino mice of either sex weighing 20-25 grams were used for the evaluation of memory enhancing activity. The animals were housed under standard environmental conditions of temperature and humidity (25±0.5° C) and (12 hours light and dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water ad libitum. The total 50 young male mice were employed in the present study. Each group comprised of ten animals.

Alcoholic Extract: The air-dried crude drug (500g) was pulverized and extracted with alcohol using Soxhlet apparatus for 16 hours. Alcohol removal carried out under pressure afforded a semisolid mass. The extract was further used for the evaluation of memory enhancing activity.

Behavioural methods for testing learning and memory by Scopolamine induced amnesia

Group I: Control group for young mice. Food was administered orally for 14 consecutive days. TL was recorded after 90 minutes of food administration.

Group II: Positive control for young mice. Piracetam (140 mg/kg i.p) injected to young mice. TL was recorded after 60 minutes of injection.

Group III: Scopolamine (0.4 mg/kg i.p) was injected on 12th day to young mice and TL was recorded 45 minutes after injection. Retention was examined after 24 hours.

Group IV and **V**: Hydroalcoholic extract (120-240 mg/kg respectively p.o, scopolamine 0.4 mg/kg i.p) were administered orally along with diet and TL was recorded after 90 minutes.

Behavioural methods for testing learning and memory by Sodium nitrite induced amnesia

Sodium nitrite induced amnesia is a type of Interoceptive aversive stimuli model[7]. Sodium nitrite (35 mg/kg, s.c.) was administered 45min before the learning trial on day 14 to induce amnesia in mice. Apparatus and procedure was same as step down passive avoidance test.

Design of the experiment

Species - Mus musculus and Strain - Swiss albino, Sex –Male, Body weight- 25-30 g

Mice were divided into 5 groups of 10 animals each.

Group 1: Distilled water (0.2 ml p.o.)

Group 2: Sodium nitrite (35 mg/kg s.c.)
Group 3: Piracetam (140 mg/kg p.o.) and Sodium nitrite (35 mg/kg s.c.)
Group 4: AC (120 mg/kg p.o.) and Sodium nitrite (35 mg/kg s.c.)
Group 5: AC (240 mg/kg p.o.) and Sodium nitrite (35 mg/kg s.c.)

Step down passive avoidance

Passive avoidance response is extensively used for the screening of drugs effecting learning and memory. The test involves training of mice to avoid punishment (normally an electric shock) by curbing a normal behaviour (such as an exploratory behaviour). At specified interval after training, the animals were tested again for retention of such learning.

Apparatus

The passive avoidance apparatus consists of a Plexiglas box (30cm x 30cm x 35cm) with a steel rod grid floor (29 parallel steel rods, 0.3cm in diameter set 1cm apart). A wooden platform (4cm x 4cm x 2cm) was placed in the centre of the grid floor. Intermittent electric shocks (0.5 mA, 10s, 30V DC) were delivered to the grid floor by an isolated stimulator.

Procedure: A typical paradigm consists of three phases. Day 1 to 13 drug *Acorus calamus* was administered orally once a day with distilled water.

Phase 1 Familiarization

Day 13 The drug *Acorus calamus* was administered 90min before placing the animal on wooden platform. The animal was placed on the wooden platform situated in the centre of the passive avoidance box and the latency to step down was recorded. After 10s of exploration, it is returned to the home cage. Animals showing step down latency (SDL) of more than 12sec in the first training session were excluded from the experiment.

Phase 2 Learning: Day 14 (24 hr retention interval) The drug *Acorus calamus* was administered 90min before the admonition of scopolamine (0.4 mg/kg i.p.). 45min after scopolamine mice were placed on wooden platform. Immediately after stepping down the animal received unavoidable foot shock (0.5 mA, 10s, 30V DC). The animals were then returned to its home cages.

Phase 3 Retention test

On the following day 15 (24 hr retention interval). Administered the drug *Acorus calamus* 90 min before placing the animal on wooden box and the step down latency was recorded. Electric shock was not administered at this time. If the mouse remained on the platform for the 5min test duration, it was assigned a maximum score of 300s.

RESULTS

Step down passive avoidance

1. Scopolamine induced amnesia

Scopolamine (0.4 mg/kg b.w) has induced marked cognitive deficit in mice as indicated by decrease in SDL during retention (15^{th} day) trials. Effect of Piracetam and hydroalcoholic extract of *Acorus calamus* on step down latency (SDL) of cognitive deficit mice by using step down apparatus. The results are summarized in Table-1 to indicate that the dose of AC Dose 1 (120 mg/kg b.w), AC Dose 2 (240 mg/kg b.w) and Piracetam (140 mg/kg b.w) produced a significant increase in step down latency (SDL) in mice on 15th day when compared to Scopolamine (0.4 mg/kg b.w) induced amnesia. Significant effect produced by AC Dose 1($P<0.05$), AC Dose 2 ($P<0.05$) and Piracetam ($P<0.001$) (Fig.1).

2. Sodium nitrite induced amnesia

Sodium nitrite (35 mg/kg b.w) has induced marked cognitive deficit in mice as indicated by decrease in SDL during retention (15th day) trials. The results are summarized in Table-2; graphical representation was shown in Fig.2. The results are summarized in Table 5.2a indicate that the dose of AC Dose 1 (120 mg/kg bow), AC Dose 2 (240 mg/kg b.w) and Piracetam (140 mg/kg b.w) produced a significant increase in step down latency (SDL) in mice on 15th day when compared to Sodium nitrite (35 mg/kg b.w) induced amnesia.

AC Dose 1($P<0.05$), Dose of AC Dose 2 ($P<0.01$) and Piracetam ($P<0.05$), significantly reversed the hypoxic deficits of retention (15th day) trials.

Table-1: Effect of Piracetam and hydroalcoholic extract of *Acorus calamus* on step down latency (SDL) of cognitive deficit mice by using step down apparatus

Group no	Treatment	SDL on 15th Day(sec)
I	Control (distilled water)	17.90 ± 1.89
II	Scopolamine (0.4 mg/kg b.w)	3.9 ± 0.526 *a
III	Piracetam(140 mg/kg b.w) +Scopolamine (0.4 mg/kg b.w)	19.20 ± 5.43 *b
IV	AC Dose I (120 mg/kg)+Scopolamine (0.4 mg/kg b.w)	18.20 ± 3.10 *b
V	AC Dose II (240mg/kg)+Scopolamine (0.4 mg/kg b.w)	33.0 ± 5.56 ***b

Data are mean ± SEM values, n=10. Data were analyzed by One way ANOVA followed by Dunnett's Multiple Comparison Test. * P < 0.05, ** P < 0.01, *** P<0.001.
[a-] compared with control,
[b-] compared with scopolamine induced amnesia

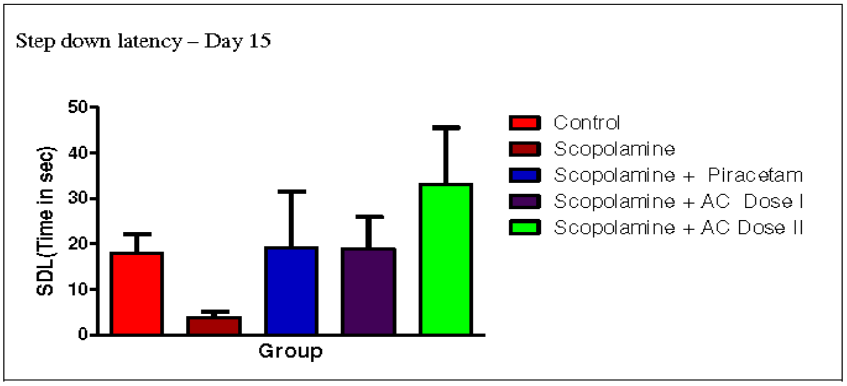

Fig.1. Effect of Piracetam and hydroalcoholic extract of *Acorus calamus* on step down latency (SDL) of cognitive deficit mice by using step down apparatus

Table.2 Effect of Piracetam and hydroalcoholic extract of Acorus calamus on step down latency (SDL) of cognitive deficit mice using step down apparatus

Group No	Treatment	SDL on 15th Day (sec)
I	Control (distilled water)	14.40 ± 2.56
II	NaNO2 (35 mg/kg b.w)	4.10 ± 0.64 *a
III	Piracetam (140 mg/kg b.w)+NaNO2 (35 mg/kg b.w)	15.00 ± 3.26 *b
IV	AC Dose I (120 mg/kg b.w)+NaNO2 (35 mg/kg b.w)	16.30 ± 3.34 **b
V	AC Dose II (240 mg/kg b.w)+NaNO2 (35 mg/kg b.w)	14.40 ± 1.49 *b

Data are mean ± SEM values, n=10. Data were analyzed by One way ANOVA followed by Dunnett's Multiple Comparison Test. * P < 0.05, ** P < 0.01, *** P<0.001.
[a-] compared with control,
[b-] compared with Sodium nitrite induced amnesia.

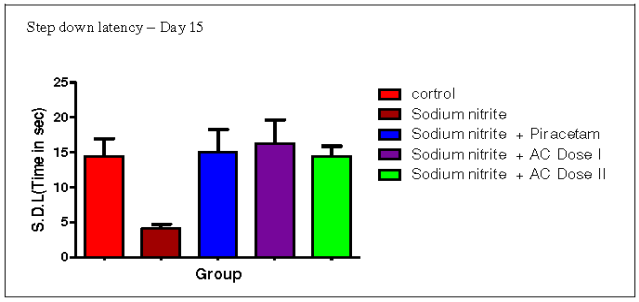

Fig.2.Effect of Piracetam and hydroalcoholic extract of Acorus calamus on step down latency (SDL) of cognitive deficit mice using step down apparatus.

DISCUSSION

Anxiety is unpleasant feeling of apprehension or fearful concern. It can be a normal, reasonable and expected response to a stressful situation or perceived danger or it may be an excessive, irrational state that signifies a mental disorder. Nootropics are a class of psychotropic agents with selective facilitatory effect integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory[8,9]. Piracetam, the first representation of a class of nootropic agents, as been shown to improve memory deficits in geriatric individuals. Repeated injections of Piracetam had improved learning abilities and memory capacities of laboratory animals[10]. The data shown by experimental results underlined the importance of hydroalcoholic extract of *Acorus Calamus* is preventing memory loss. New neurons are continuously being added to certain areas of brain such as hippocampus and olfactory bulb in animals as well as humans[11]. There is a possibility that hydroalcoholic extract of *Acorus Calamus* for long periods not only arrest the neurodegenerative process but also stimulated the process of neurogenesis. The elevated plus maze is a well established animal model for testing anixolytic drugs. Considering the lack and need of drugs with proven effectiveness in improving learning and memory, the specific memory improving effect of *Acorus Calamus* reported here is of enormous interest and deserves further investigations using more experimental paradigms for further confirmation of memory improving potential of *Acorus Calamus* in the treatment of various cognitive disorders. Acorus calamus is known as "Vacha" in Ayurveda, which have several medicinal uses as mind care, anti-ulcer, anti-spasmodic, analgesic, anti-inflammatory, anti-convulsion and anti-bacterial activity. Memory enhancer agents are known to facilitate learning and memory, and prevent impairment of cognitive functions induced by diseases and brain insults. There is extensive experimental evidence that Piracetam and its analogues can facilitate learning acquisition and memory in a variety of animal models of impaired cognitive function including learning deficits in aged animals.

Step down passive avoidance:

Passive avoidance is based on the negative reinforcement and is used to examine long term memory, where in the animal learn to avoid noxious events by suppressing its normal exploratory behaviour. Scopolamine administration in animals affects several aspects of short term memory and attention leading to cognitive deficits as observed during the acquisition and retrieval phase which is similar to ageing and dementia patients. Scopolamine blocks the muscarinic receptors involved in the modulation of memory leading to decreased attention and learning abilities.

Scopolamine administration in Scopolamine group animals statistically significant decreases the SDL compared to control animals. SDL for control animals on day 15 was (17.90sec) and for scopolamine SDL was (3.90sec). This clearly indicates reduction in the memory after administration of scopolamine. SDL for piracetam (19.20sec), AC Dose 1 (18.20sec) and for AC Dose 2 was (33.00sec). This indicates the increase in SDL compare to

scopolamine. Piracetam and AC Dose 1 compares well with control SDL. This indicates that piracetam and AC Dose 1 bring animals to normal stage or it nullifies the effect of scopolamine. SDL for AC Dose 2 indicate that the effect of Scopolamine not only nullify but also was better than the control animals memory. Hydroalcoholic extract of Acorus calamus Dose 1 (120 mg/kg b.w), Dose 2 (240 mg/kg b.w) and Piracetam (140 mg/kg b.w) treated animals were increased SDL values on 15th day. It indicates animals were able to retain the learned activity.

Sodium nitrite induced amnesia:

Chemical hypoxia induced by administration of sodium nitrite, resulting in the reduction of oxygen carrying capacity of blood with conversion of haemoglobin to methemoglobin. During investigation of Sodium nitrite on brain metabolism, demonstrated a close relationship between oxidative metabolism and cholinergic function. Sodium nitrite administration in Sodium nitrite group of animals show statistically significant decrease in the SDL compared to control animals. SDL for control animals on day 15 was (14.40sec) and for sodium nitrite SDL was (4.10sec). So it was clear indication to reduce the memory after administration of sodium nitrite. SDL for piracetam (15.00sec), AC Dose 1 (16.30sec) and for AC Dose 2 was (14.40sec). It indicates the increase in SDL compare to sodium nitrite. Piracetam, AC Dose 1 and AC Dose 2 were as similar compare to control SDL. So it indicate that piracetam, AC Dose 1 and AC Dose 2 bring the animals to normal stage or it nullify the effect of sodium nitrite. Sodium nitrite (35 mg/kg, b.w. s.c.) has induced marked cognitive deficit in mice as indicated by decrease in SDL on 15th day. Piracetam (140 mg/kg b.w), A C Dose 1 (120 mg/kg b.w) and A C Dose 2 (240 mg/kg b.w) increased SDL on 15th day statistically significant showing reversal of sodium nitrite induced amnesia.

CONCLUSION

Conclusion in the light of above, it may be worthwhile to explore the potential of hydroacloholic extract of *Acorus Calamus* exhibited nootropic activity and useful in management of Alzhemer's disease in suitable formulation.

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