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# Therapeutic Approaches for Cognition Enhancement

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***Abstract:*** **Cognitive disorders such as amnesia, attention deficit and Alzheimer's disease are emerging nightmares in the field of medicine because no exact cure exists for them, as existing nootropic agents has several limitations. The present study was undertaken to evaluate the combination of extracts of *Acorus calamus* and *Panax ginseng* in different ratios for cognition enhancement.**

**The combination (1:1) and (2:1) was administered orally at different doses for 14 consecutive days in Male Wistar rats. Aricept (5 mg/kg) as used as a standard nootropic agent. Learning and memory parameters were evaluated using elevated plus maze(EPM) and novel object recognition model(NOR).Brain AChE activity was also evaluated.It was observed that amongst the two ratios (1:1) and (2:1), the ratio (1:1) was found to give more significant results than the other by significantly reversing the scopolamine induced amnesia(0.5mg/kg), as evidenced by a decrease in the transfer latency in the EPM task and increase in the recognition index in NOR task. The combination (2:1) was found to reduce brain AChE activity more significantly than (1:1).**

***Acorus Calamus*** **being Acetyl cholinesterase inhibitor and *Panax ginseng* being NMDA receptor antagonist. Both the plants possess different signaling cascade. These plants act via two different, but interconnected, pathological pathways, and that their complementary activity may produce greater effects than either drug individually. Also, diminished cholinergic transmission appear to be responsible for the development of amyloid plaques and dementia in Alzheimer's patients, the combinations used may prove to be a useful memory-restorative agent. Also, the combination (2:1) would be worthwhile to explore the potential of these plants in the management of Alzheimer's disease as compared to combination (1:1).**

***Keywords:*** **Alzheimer's disease, amnesia, Acorus calamus, Panax ginseng.**

## 1. INTRODUCTION

Cognition enhancement refers to the improvement of cognitive ability in normal healthy individuals. However, the term cognition enhancement is usually used in connection with interventions applied more directly to the brain. The cognitive disorder syndrome is delirium and dementia commonly are associated with definite neuropathological, metabolic or toxic (including drug induced) changes and are characterized by confusion, disorientation and memory disturbances as well as behavioral disorganization. In general, the effectiveness of pharmacological treatment core cognitive impairment in dementia remains limited despite extensive effective treatment. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), Down's syndrome.(1)

Alzheimer's disease is a progressive neurodegenerative disorder that is slow in onset but ultimately leads to dementia. Formation of memory is the most complex process and involves multiple neuronal pathways and neurotransmitters. It is well known that the cholinergic neuronal system plays an important role in learning and memory in humans and animals. This is the therapeutic rationale behind the use of of nootropic agents such as piracetam, various piracetam analogues etc.The Indian system of medicine is replete with medicinal plants claimed to promote learning,memory and intelligence.(2)

There are several approaches for management of cognition. Herbal medicines have proved to be highly beneficial for its use as complementary or adjunct therapy. In several cases, it has proven efficacious even when used as a monotherapy. Several herbs are used alone or in conjugation with other herbs possessing different mechanism of action for the management of disease. Thus a polyherbal formulation can prove to be highly efficacious due to the additive effect of its constituents.

In development and increasing the use of traditional medicines, folklore knowledge has been one of the greatest assets. Based on this knowledge several herbs are used alone as well as in combination for management of the disorder. Various plants used in the management of memory disorder *are Allium satium, Bacopa moneri, Celatrus Paniculatus, Nicotiana tobacum, withania somnefera, etc.*

The plant *Acorus calamus* commonly known as sweet flag is a perrinaial herb which grows in mainly in swamps, marshes and river banks. In aurvedic medicine, the rhizome has been used for treatment of memory loss. Two rhizome extracts ethanolic and hydroethanolic exerted sedative and neuroprotective effect in vivo respectively. (3)Also, the plant *Panax Ginseng* roots has been reported for the improvement of learning and memory in various animal models. Both these plants are found to possess various pharmacological properties as well (4). In the light of the above, the present study was undertaken to investigate the effect of *Acorus Calamus* and *Panax Ginseng* in combination of (1:1) and (2:1) on cognitive function and brain cholinesterase(ChE) activity in scopolamine –induced amnesia in rats.

## 2. MATERIALS AND METHODS

**Animals:**

Male Wistar rats strain was used in this study.Their weights were in the range 180-200g.The rats were allowed free access to food and water. Animals were housed in groups of three to four per cage and were kept under controlled room temperature (24±2degree Celsius) in a 12-h light-dark cycle. The experiment was conducted on a noise-free experiment between 8.00 and 12.00 am. Prior approval was obtained from the Institutional Animals Ethics Committee which followed guidelines of CPCSEA, for conducting the study.

**Acute Oral Toxicity:**

The acute toxicity was performed according to the OECD 423 guidelines. The combination at the dose of 300, 2000 and 5000mg/kg body weight, was administered to the rats and they were subsequently observed closely for the first 4 hours for any untoward symptoms such as tremors, convulsions, salivation, diarrhea and lethargy followed by observation for a further 14 days. At the end of the experiment period, the animals were observed for any changes in behavioral pattern and mortality.

**Chemicals:**

The chemicals used in the study were Scopolamine hydro bromide (NMIMS), *Panax ginseng* extract (Navchetnakendra, shastrinagar, New delhi), *Acorus calamus* rhizomes (Yucca enterprises), ethanol, acetylthiocholine iodide, sodium dihydrogen phosphate, disodium hydrogen phosphate(Loba chem, Mumbai).

**Drug administration:**

*Acorus Calamus* rhizomes were purchased from yucca enterprises and were authenticated at Botany department of Mithibhai College, Mumbai and then Soxhlet extraction was done using hydro alcoholic solvent. *Panax ginseng* extract was purchased from Navchetnakendra enterprises, shastri nagar, New Delhi.

**Pilot study individually for both the drugs:** Initially, pilot study was done for both the herbal drugs (*ACORUS CALAMUS AND PANAX GINSENG*) individually starting with the dose of 50mg/kg, then 100mgkg and 200mg/kg consisting of 6 animals per dose. From the literature review and the study performed it was seen that for *Acorus calamus* more significant result was obtained at 100mg/kg and it was seen that there is little significant difference between the doses 100mg/kg and 200mg/kg for *Acorus calamus.*

For *Panax ginseng* also, 100mg/kg showed more significant result than the other two doses 50mg/kg and 200mg/kg.

**MAIN STUDY:** To look for the approaches for cognition enhancement, herein two approaches were taken based on the pilot study. Firstly, 100mg/kg for *Acorus calamus* and 100mg/kg for *Panax ginseng* in the ratio (1:1) .Then, as seen from

the pilot study less significant difference was seen between the dose for *Acorus calamus* at 100mg/kg and 200mg/kg and to further evaluate the results, second approach was taken as *Acorus calamus* at 200mg/kg and *Panax ginseng a*t 100mg/kg in the ratio (2:1).

The animals were then divided into 7 groups with each consisting of 6 rats. All the animals were given access to food and water ad libitum. The following table enlists the groups and the treatment given to each group:

| Group no | Group | Animal Model | Evaluation |
|---|---|---|---|
| I | Control Distilled water( 5ml/ kg, p.o ) | 1. Elevated plus maze | Transfer Latency, Inflexion ratio |
| II | Scopolamine (0.5 mg/ kg, ip) **(Negative control)** | | |
| III | Aricept (5 mg per kg p.o) + Scopolamine (0.5 mg/ kg, ip)**(Standard)** | | |
| IV | *Acorus calamus* (100 mg/kg , p.o) + Scopolamine (0.5 mg/ kg, ip)(**TEST 1)** | | |
| V | *Panax ginseng* ( 100 mg/kg, p.o) + Scopolamine (0.5 mg/ kg, ip) **(TEST 2)** | 2. Novel object recognition | Recognition Index |
| VI | *Acorus calamus* + *Panax ginseng* (1:1) + Scopolamine (0.5 mg/ kg, ip) **combination 1** | | |
| VII | *Acorus calamus* + *Panax ginseng* (2:1) + Scopolamine (0.5 mg/ kg, ip)**combination2** | | |

Dosing was done for 14 days at the same time (i.e.,8.00-9.00am) on each day.

**Experimental design:**

The experimental design was planned such that the effect of the all the groups could be evaluated after 14 days against scopolamine-induced amnesia.After the treatment period of all the groups for 14 days, all the animals were subjected to scopolamine (0.5mg/kg) 60mim after the drug administration, except the first group which served as vehicle control.The cognitive paradigms were evaluated 45min after the scopolamine administration using the elevated plus maze and novel object recognition model.

**Elevated plus maze:**

Elevated plus maze served as the exteroceptive behavior model to evaluate learning and memory in rodents. The procedure, technique and end point for testing, learning and memory are followed as per the parameters described by the investigators working in the area of neuropsychopharmacology. The apparatus consisted of two open arms and two enclosed arms. The arms extended from a central platform and the maze is elevated to a height of 25cm from the floor. On the 14 day, each mouse is placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is the time taken by mouse with all its four legs to move into one of the enclosed arms, is recorded on the first day. If the animal did not enter into the one of the enclosed arms within 90 s, it is gently pushed in to one of the two enclosed arms and the TL is assigned as 90 s. The mouse is allowed to explore the maze for another 10 s and then returned to its home cage. Retention of this learned task is examined 24 h after the first day trail ($15^{th}$ day). The inflexion ratio was calculated by the formula IR = Lo – Lt/Lo , where Lo is initial TL on first day and Lt is TL after 24 hours. Decrease IR indicates the induction of amnesia, and increased IR indicates in improvement in cognition and memory impairment. (5)

**Novel object recognition:**

Object recognition may be performed in a simple box, with or without a transparent wall. Procedure consists of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the 1st day (habituation phase), rats were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 2nd day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size). The two objects, made of the same material with the similar color and smell, were different in shape but identical in size. Rats were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats. On the 3rd day (retention phase), rats were allowed to explore the open

field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index (for retention session), calculated for each mouse, was expressed as the ratio (TC×100)/(TA + TC), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring any object (nose pointing toward the object at a distance≤1 cm, but not mounting on the object or playing with the object) was recorded.(6)

**Estimation of Ach Levels in the brain:**

After assessing the learning and memory paradigms in scopolamine induced amnesia, rats from each group were euthanized by cervical decapitation; brains are removed quickly and placed in ice-cold saline. Frontal cortex, hippocampus and septum (and any other regions of interest) are quickly dissected out on a petri dish chilled on crushed ice. The tissues are weighed and homogenized in 0.1M Phosphate buffer (pH 8). 0.4ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a UV spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 mins at intervals of 2 min. Change in the absorbance per minute is thus determined. (7)

## 3. RESULTS

**Acute oral toxicity profile:**

The rats were treated with the combination *(Acorus calamus and Panax ginseng*) taken at doses 300, 2000 and 5000mg/kg, p.o exhibited normal behavior. They were alert with normal grooming, touch response and pain response. There were no signs of passivity, stereotypy and vocalization. The animals showed no signs of depression. Alertness, limb tone and grip strength as well as the gait of the animals were normal. The combination was found safe upto 5000mg/kg in rats and the oral administration of these extracts was non-toxic. (Table no-1)

**Elevated plus maze:**

The effect of all the drug treated groups was evaluated at the end of 14 day. Transfer Latency was recorded. It was seen that Transfer Latency for all the drug treated groups was less on 15$^{th}$ day as compared to 14$^{th}$ day except the negative control group. The effect of TL was expressed as inflexion ratio. Decrease IR indicates the induction of amnesia, and increased IR indicates in improvement in cognition and memory impairment. The inflexion ratio of negative control group(scopolamine) animals were significantly($p<o.oo5$)decreased in comparison with all the groups that indicated that amnesia is induced.Also,the inflexion ratio of combination1 showed significant increase in comparison with test1 and test2 individually and the inflexion ratio of combination2 showed significant increase in comparison with test2.(Table no.2 and fig1 and 2).

**Novel object recognition:**

The exploring time for novel object expressed as recognition index. Increase in recognition index (RI) indicates nootropic activity. From, the table3 we can see that all the animals in all the groups spent more time in exploring the novel object except the negative control(Scopolamine) group. The RI of the control group was 51.61±1.8%.Scopolamine showed significant decrease in recognition index as30.645±3.2%. The combination1 showed significant ($P<0.05$) increases in the novel object exploration indicated as increased recognition index in comparison with test1 and test2.The combination2 shows significant increase when compared with vehicle control and negative control group. The standard and the treatment groups antagonize the effects of scopolamine by increasing novel object exploration time. (Table no.3 and fig 3 and 4)

**Estimation of brain AChE level:**

All the groups showed decreased acetlcholinesterase enzyme activity as compared with scopolamine as indicative in table 4. The acetylcholinesterase activity was significantly increased by scopolamine as compared to control. The increase in AChE activity by scopolamine was significantly reduced by drug treated gropus.Combination1 showed significant decrease in acetylcholinesterase activity in comparison with test2, whereas, combination2 showed significant decrease in acetylcholinesterase enzme activity in comparison with test1 and test2 both.

**Table 1: Acute oral Toxicity results of combination**

| Organ | Observations |
|---|---|
| Liver | Mild to moderate degree glycogen infiltration minimally accentuated in periportal zones |
| Kidney | No abnormalities were detected |
| Heart | No abnormalities were detected |

*Remarks : Lesions suggestive of toxicity were not noted(non-toxic).*

**Table No 2: Effect of treatment groups on inflexion ratio(elevated plus maze) in scopolamine induced amnesia in rats)**

| TREATMENT GROUPS | TRANSFER LATENCY ON 14th DAY(LO) | TRANSFER LATENCY ON 15th DAY(Lt) | INFLEXION RATIO(LO-LT/LO)(mean±SEM)% |
|---|---|---|---|
| Vehicle control | 48±22.11 | 32.66±7.36 | 0.449±0.032 |
| Scopolamine hydrobromide(Negative control) | 65±33.3 | 84.23±9.77 | 0.222±0.024** |
| standard | 57.83±20.91 | 16.33±81 | 0.711±0.032*$^{?}$ |
| Test 1 | 64±16.087 | 26.16±3.43 | 0.576±0.030*$^{?}$ |
| Test 2 | 69.166±13.96 | 44.833±8.08 | 0.347±0.011**$^{?}$ |
| Combination 1 | 33.66±15.41 | 13.16±4.021 | 0.637±0.026**$^{\#@?}$ |
| Combination2 | 60.166±13.54 | 25.833±6.79 | 0.554±0.020*$^{@?}$ |

*(Values are expressed as mean±SEM AT n=6;One way ANNOVA followed by Turkey's honest test;significance is denoted by *p<0.05; **p<0.01 when compared against control, $^{\#}$p<0.05 when compared against Test1, $^{@}$ p<0.05when compared against Test2, $^{?}$p<0.05 when compared against Negative control(induction)*

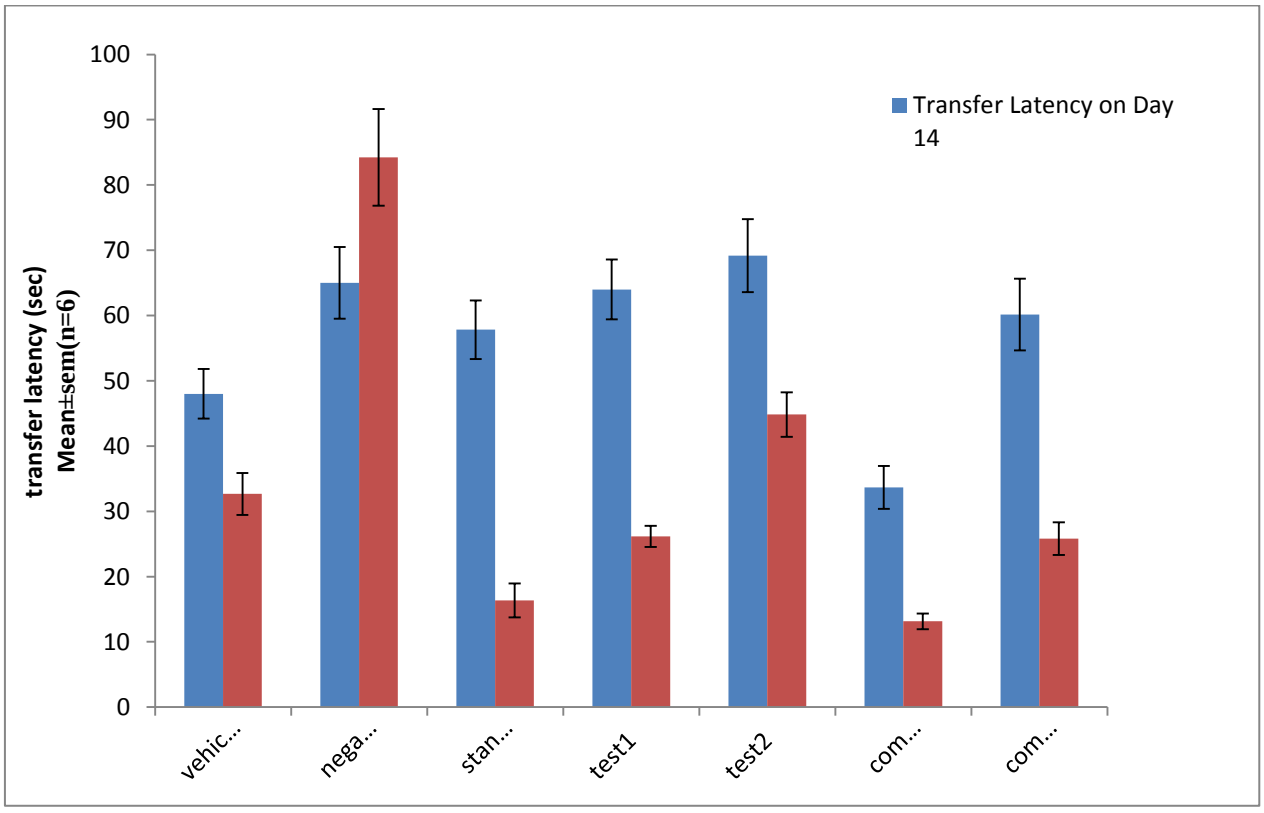


**Figure: 1 Effect of combination on Elevated Plus Maze (Transfer Latency)**

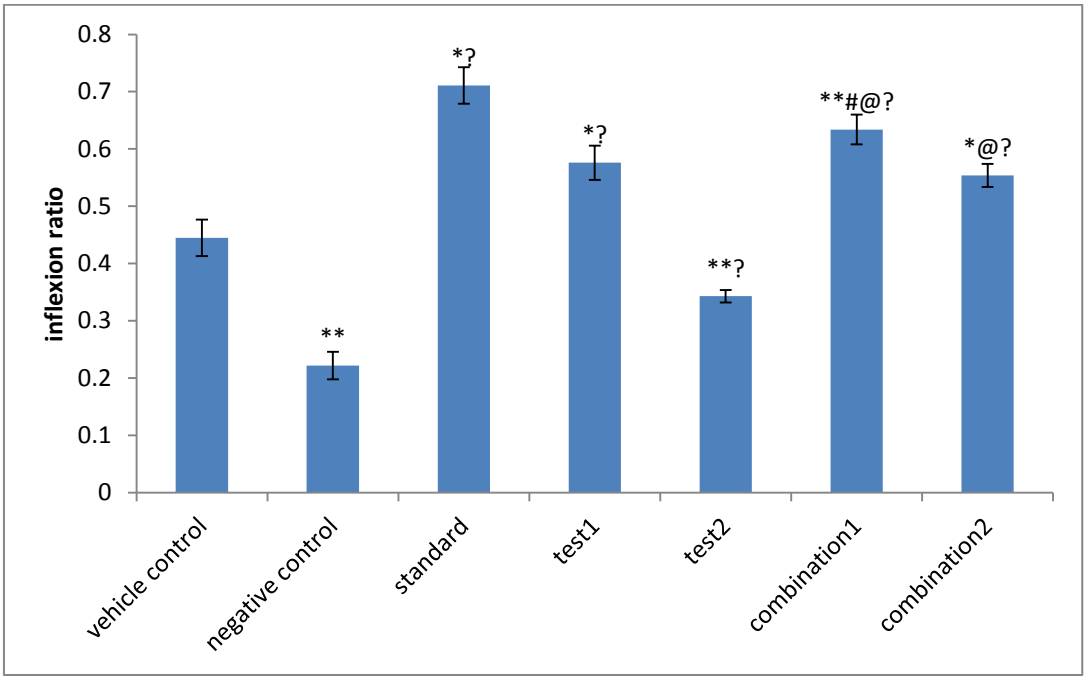


**Figure:2 Effect of combination on Elevated Plus Maze(inflexion ratio)**

**Table 3: Effect of treatment groups on Recognition index in Novel object recognition test**

| Treatment groups | Time spent Familiar object(sec) | Time spent New object(sec) | Recognition index(mean±SEM)% |
|---|---|---|---|
| Vehicle control | 10.16±0.47 | 11.16 ±1.27 | 51.61 ± 1.8 |
| Scopolamine hydrobromide(Negative control) | 32± 20.98 | 14.50 ±2.48 | 30.645 ± 3.2* |
| standard | 15.83± 2.60 | 42.83 ±2.27 | 73.913 ± 2.6**? |
| Test 1 | 17.50 ±3.22 | 37.66± 1.82 | 53.73 ±3.6*? |
| Test 2 | 20.50±2.96 | 24.83± 4.58 | 64.64 ±4.98*? |
| Combination 1 | 17± 5.29 | 28.83± 4.12 | 70.675 ±5.6*?#@ |
| Combination2 | 11± 2.20 | 20 ±2.91 | 69.36 ± 2.9*? |

*(Values are expressed as mean±SEM AT n=6;One way ANNOVA followed by Turkey's honest test;significance is denoted by *p<0.05; **p<0.01 when compared against control, #p<0.05 when compared against Test1, @ p<0.05when compared against Test2, ?p<0.05 when compared against Negative control(induction)*

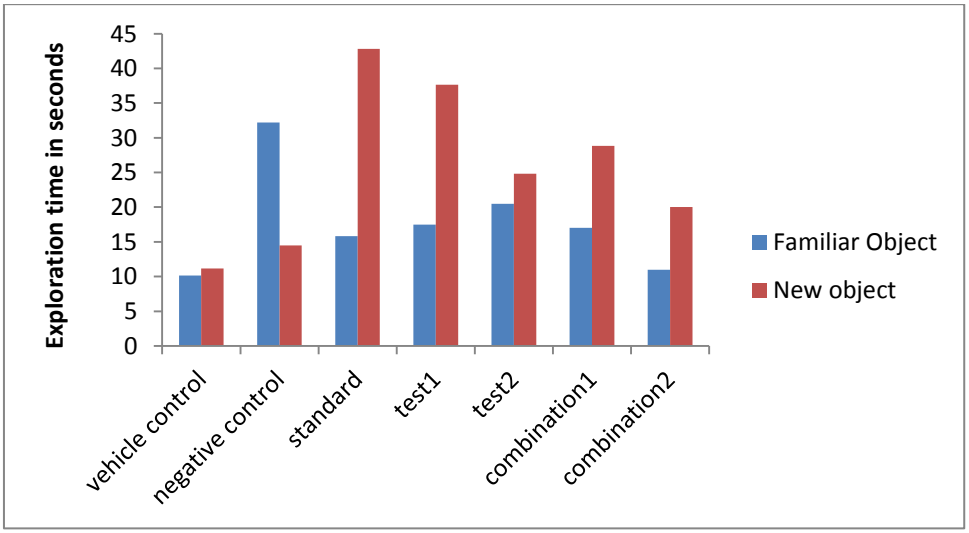


**Figure:3 Effect of combination on object recognition test.**

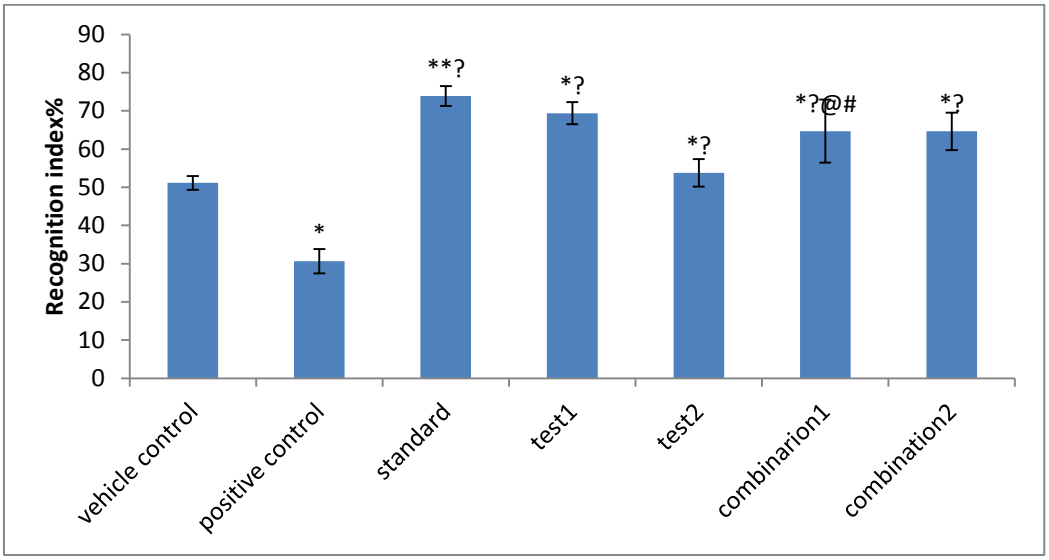


**Figure:4 Effect of combination on recognition index in object recognition test**

**Table 4: Effect of combination on acetyl cholinesterase enzyme activity**

| Treatment groups | AChE enzme activity |
|---|---|
| Vehicle control | 10.38± 0.30 |
| ssssssssScopolamine hydrobromide | 23.11 ±1.3** |
| standard | 8.71± 0.30*? |
| Test 1 | 15 ±0.39*? |
| Test 2 | 18.92± 0.23*? |
| Combination 1 | 17.50± 1.1*?@ |
| Combination2 | 11±1*?@# |

*(Values are expressed as mean±SEM AT n=6;One way ANNOVA followed by Turkey's honest test;significance is denoted by *p<0.05; **p<0.01 when compared against control, #p<0.05 when compared against Test1, @ p<0.05when compared against Test2, ?p<0.05 when compared against Negative control(induction)*

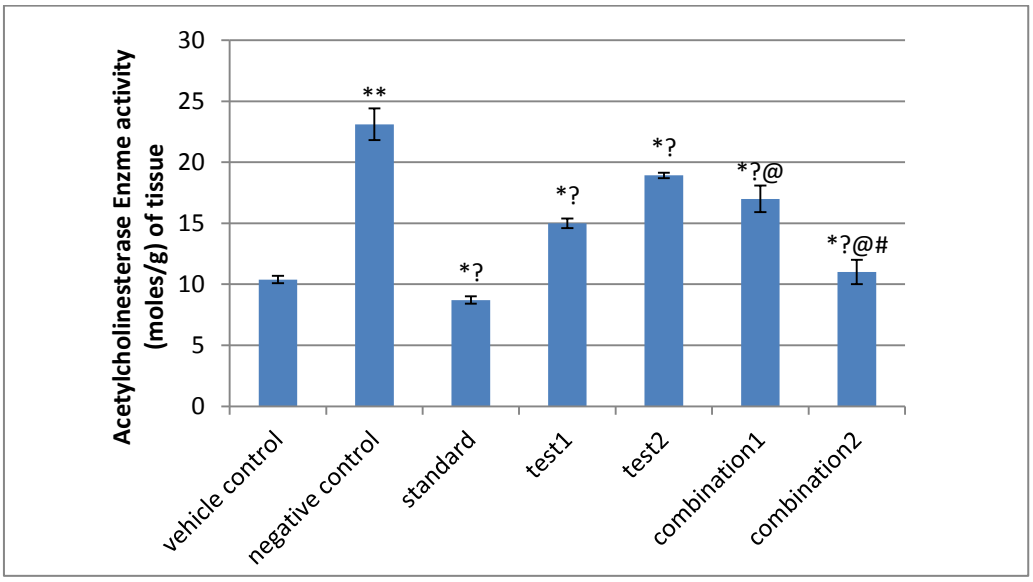


**Figure 5: Effect of combination on acetyl cholinesterase enzyme activity**

## 4. DISCUSSION

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities.(8)Despite the severity and high prevalence of this disease, the allopathic system of medicine is yet to provide a satisfactory antidote. Hence, the present study focuses on exploration of the memory enhancing activity of the combination of *Acorus calamus* and Panax *ginseng* in 1:1 and 2:1 in a scopolamine induced amnesia rat model.

The present study suggests that the combination possess memory enhancing activity in view of its facilitator effect on the retention of spatial memory in scopolamine-induced amnesia. There is decrease in the TL i.e. rats were able to locate the dark zone immediately after exposure to the open arm in the EPM paradigm, which is an indicator of cognition improvement. Also, more satisfactory results were obtained from combination1.

The object recognition test suggests that spontaneous tendency of rodents to explore the novel object (situation) in the test trial based on memory of previous experience from the acquisition trial. In present study the object recognition index is determined with rats and demonstrate its characteristic as spatial memory work . Rats treated with combination1and 2 after experiencing an acquisition trial spent more time exploring the novel object. Thus from results and observation the present study indicated that the combination used possesses nootropic activity. Also, results of combination1 were more significant as compared to test groups.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic transmission is terminated by acetylcholine hydrolysis via enzyme Acetylcholinesterase (AChE). This enzyme is essential in maintaining normal function of nervous system, since it rapidly terminates the action of acetylcholine released into the synapse. It is believed that the action of this enzyme could affect the underlying processes in AD. Consequently, AChE has been potential target for prevention and treatment of Alzheimer's disease. Marked reduction in acetlcholinesterase activity was seen in combination2.

From the above results it was seen that Combination1 showed satisfactory results from performed behavioral models, whereas Combination2 results were more acceptable as Acetylcholinesterase enzyme activity showed marked reduction in comparison with test1 and test2. The probable active constituent in Acorus calamus is β asarone which is known for its acetylcholinesterase activity. So, when taken in combination of (2.1) *Acorus calamus* (2) and *Panax ginseng* (1), the increased concentration of this oil in the extract may be responsible for nootropic activity.(8)

*Acorus Calamus* being Acetylcholinesterase inhibitor(active constituent-βasarone) and *Panax ginseng* (active constituent-ginsennosides) being NMDA receptor antagonist possess two different signalling cascade in Alzheimer's diseases. The proposed mechanism of action would be that NMDA Receptor antagonist and the AChEIs intervene at separate points of the disrupted signalling cascades in AD. Acting at the NMDA receptor,*Ginseng* might lower the pathologically increased tonic level of excitation of the glutamatergic synapse at rest . This is likely to have a two fold impact: firstly, it reduces the background noise, so that incoming physiological signals can be better distinguished; secondly, it reduces the constant pathological influx of Ca2?, and thereby helps to prevent the neurone being stimulated in a way that would cause both dysfunction, synaptotoxicity and ultimately cell death (neurone 2) . Overall, tonic NMDA receptor activation is reduced, which delays the neurodegeneration of cholinergic neurones bearing NMDA receptors, and synaptic NMDA receptor activation is facilitated . Supplementing this effect, the AChEIs may serve to amplify (i.e. bring towards normal) the pathologically weakened signal from cholinergic neurons by delaying ACh breakdown at cholinergic nerve ending. In this way, neurotransmission is preserved, with the improved signal detected against the lowered background noise. Together, such effects would help to maintain the glutamatergic/cholinergic signalling cascades, and consequently facilitate LTP(long term potentiation) and memory processes. In practice, glutamatergic neurones not only make synaptic connections with cholinergic neurones, but

cholinergic neurones also influence glutamatergic transmission in areas such as the cortex and hipppocampus.

Therefore, applied together, NMDA receptor antagonist and AChEIs have the potential to act at different places in interconnected pathways, with complementary mechanisms potentially producing additive effects opposing disease pathology. In addition to this restoration of function,NMDA receptor antagonist also appears to protect against excitotoxicity and therefore consequent neurodegeneration.

Central system plays an important role in learning and memory (10). Anti-cholinergic drugs like scopolamine impair the learning process and negatively affect the memory performance (11). Memory impairment in the patients with senile

dementia of Alzheimer's type results from a deficiency in cholinergic function in the brain. Present investigation demonstrates the effect of *Acorus Calamus* and *Panax ginseng* in combination on cholinergic system. The observation shows that combination has antagonised the amnesic effects of scopolamine, improvement in learning, memory and cognition on the EPM and Object Recognition Test. This indicates the action of Combination on cholinergic system, as it has long been known that the stimulation of the cholinergic system improves cognitive processes. But further study to conform its exact mechanism is essential. Thus, it is concluded that the Combination of Acorus Calamus and Panax Ginseng, possessed nootropic activity and also indicate the involvement of central cholinergic system in this mechanism.(12)

## 5. CONCLUSION

In the present study, it was observed that *Acorus calamus* and *Panax ginseng* in combination possesses protective activity from the loss of memory and cognition deficits at different ratios. Also,caused reduction in AchE levels of brain thus suggesting anti-cholinesterase activity of the combination. In the light of the above discussion, it may be worthwhile to explore the potential of these plants in prevention, treatment and management of Alzhemier's disease.

## ACKNOWLDGEMENT

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