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Modulation of working memory by mentat and donepezil using scopolamine induced amnesia in rats.

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ABSTRACT

To compare the effect of mentat and Donepezil was on working memory in rats. The rats were trained for conditioned avoidance response by using Cook's pole climbing apparatus. Rats trained for CAR received scopolamine (hydrobromide) in a dose of 0.5mg/kg by intraperitoneal route before administration of study drugs. This is known to produce amnesia which will be used to evaluate the effect on learning and memory of study drugs. Donepezil was given in a dose of 0.32mg/kg by intraperitoneal route and mentat was given in a dose of 200mg/kg by oral route for eight days in the animals after training for conditioned avoidance response. Data was analysed by the chi-square test. Findings show that administration of single dose of scopolamine 20 minutes before the test run on the apparatus causes transient amnesia daily for resulting in disruption of the retention of CAR. Mentat and donepezil significantly increased the retention of condition avoidance response in comparison to scopolamine. However there was no significant statistical difference in the increase in the retention of CAR in mentat or donepezil given alone, but the combination of the two drugs showed statistically significant increase in the retention of CAR than either of these drugs alone. Results from this study provide good platform to conduct clinical studies to assess the benefit of combining mentat with donepezil when given for a longer period of time.

Key Words: Working memory, mentat, donepezil, scopolamine, conditioned avoidance response.

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INTRODUCTION:

Memory, one of the most complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay. Memory has been classified into several types depending upon the duration for which the information can be recalled. We have sensory memory lasting for few seconds, short-term memory, lasting for few hours and long term memory, where in the information is stored for several years or even for life time¹.

Working memory is the ability to maintain or hold temporary active representations of information for further processing or recall. The process is thought to have two components, short term storage and executive processes that operate on the stored material. Working memory is mediated by a widely distributed neural system in the human brain. Functional brain imaging studies of human have identified cortical regions that are involved in working memory including occipital, temporal, parietal and prefrontal cortical areas². These memory fields may be modulated by many neurochemical systems, including dopamine, serotonin, noradrenaline, acetylcholine, GABA and glutamate in highly differentiated ways, suggesting that modulation of these neurochemical systems may affect the different stages of working memory^{3,4}. Working memory system thus enables goal-directed behaviours, such as decision making and learning, to utilize and manipulate information beyond its transient sensory availability.

There are number of clinical conditions which can affect cognition. These are depression, schizophrenia, multiple sclerosis, vasculitis, Alzheimer's disease (AD), Parkinsonism and alcoholism but commonest is AD. AD is the most common cause of dementia in the elderly, with a prevalence that doubles every 5 years after the age of 60.

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss, altered behaviour and signs of cortical disconnection including apraxia, aphasia and agnosia⁵. Older people with dementia exist in nearly every country in the world. WHO projections suggest that by 2025, about three-quarters of the estimated 1.2 billion people aged 60 years and older will reside in developing countries. The annual healthcare costs of AD in the United States have been estimated to be as high at \$100 billion In addition to the impact on healthcare budget, there is also the emotional as well as physical stress brought to the family of the AD patient⁶.

Donepezil was the first cholinesterase inhibitor to be licensed in the UK for Alzheimer's disease. The AD2000 Collaborative Group did a study to determine whether it produces worthwhile improvement in non-cognitive and behavioural symptoms, and is a cost-effective treatment.

While the drug produced small improvements in cognition and activities of daily living in patients with this disease, no measurable reduction in rate of institutionalisation or progress of disability was recorded. The investigators concluded that donepezil is not cost effective, with benefits below minimally relevant thresholds, and clinicians should question routine prescription of cholinesterase inhibitors⁷.

Mentat is a herbal formulation of several Indian medicinal plants, which have been categorized in Ayurveda, the ancient Indian system of medicine, as Medharasayanas used to improve memory and cognitive deficits associated with chronic illness and aging. Clinical studies with Mentat have shown that it improves memory quotient in normal subjects of different age groups, increases memory span and attenuates fluctuations of attention in normal adults and improves learning ability in children with behavioural problems or minimal brain damage⁸. Ayurvedic formulations are generally multicomponent and are based on the concept that such combinations provide synergistic therapeutic effects and eliminate any adverse effects (Charaka)⁹.

In view of various reports indicating that supplementation with various herbal and allopathic drugs may enhance learning and memory and the fact that there are hardly any published trial reports on the comparative evaluation of various formulations used in India,[10] the present study was undertaken to evaluate and to compare the role of Mentat with Donepezil by using the established model of scopolamine to produce amnesia.

MATERIALS AND METHODS

Animals

Experimentally naïve Sprague Dawley albino rats weighing between 150 and 200 g of either sex were used. The rats were maintained under standard conditions of temperature ($25^{\circ}\text{C}\pm 5^{\circ}\text{C}$), relative humidity ($55\pm 10\%$) and a 12/12 hr light/dark cycle. The rats were fed with commercially available 'Amrut rat pellet feed' manufactured by Pranav Agro Food, Pune. Drinking tap-water supplied by Pimpri-Chinchwad Municipal Corporation was provided to the rats through the feeding bottles with stainless steel nozzle in each cage. Replenishment of food and water was done once daily. The study was approved by the Institutional Animal Ethics Committee.

Instruments, Drugs and Chemicals

Electro-convulsimeter and Cook's pole climbing apparatus were purchased from ST1 Instruments Private Limited and New Neeta Manufacturer, Pune, India respectively. Donepezil was purchased from Yashica Pharmaceuticals Private Limited, Mumbai, India. Mentat was

purchased from The Himalya Drug Company, Bengaluru, India. Scopolamine was purchased from Deccan Chemicals, Pune, India.

Conditioned avoidance response

This model was used to study the nootropic effects of mentat and donepezil. The rats were trained for conditioned avoidance response by using Cook's pole climbing apparatus. The method of Fellow and Cook was used with some modifications. Each rat was allowed to acclimatize for two minutes and was then exposed to a buzzer noise. After 5 seconds of putting on the buzzer, mild electric shocks were given through the stainless steel grid floor. The magnitude of the voltage was adequate (5-10V) to stimulate the rat to escape from the floor and climb the pole. As soon as the rat climbed the pole, both the buzzer and the foot shocking were switched off. At least 10 such trials were given to each rat at an interval of 1 min per day for 10 days. After about 10 days training schedule, most of the rats learned to climb the pole within 5 seconds of starting the buzzer, thus avoiding the electric foot shocks. Rats avoiding the foot shocks in all 10 out of 10 trials were considered to have developed conditioned avoidance response for further experiments¹¹.

Scopolamine induced disruption of memory

Rats trained for CAR received scopolamine (hydrobromide) in a dose of 0.5mg/kg by intraperitoneal route before administration of study drugs. This is known to produce amnesia which will be used to evaluate the effect on learning and memory of study drugs¹².

Study drug administration

- I) Donepezil: Donepezil was given in a dose of 0.32mg/kg by intraperitoneal route for eight days in the animals after training for conditioned avoidance response.
- II) Mentat: Mentat was given in a dose of 200mg/kg by oral route for eight days in the animals after training for conditioned avoidance response¹³.
- III) Scopolamine: Scopolamine was dissolved in 0.9% saline solution and given in a dose of 0.5mg/kg by intraperitoneal route, 20 minutes before test run on the apparatus.
- IV) Double distilled water was used as a vehicle for dissolving both the study drugs and was administered as a vehicle in the control group by oral route.

Grouping

The animals were divided into 5 different groups of 10 rats each after training for CAR. The animals received drugs by either intra-peritoneal route or oral route depending on the group.

The rats were divided into following groups:

C (n = 10): Rats received distilled water for 8 days by oral route, which was used as vehicle for study drugs and will serve as control.

S (n = 10): Rats in this group received Scopolamine 20 minutes before test run on the apparatus.

S + M (n = 10): Rats in this group received Scopolamine 20 minutes before administration of Mentat daily for 8 days.

S + D (n = 10): Rats in this group received Scopolamine 20 minutes before administration of Donepezil daily for 8 days.

S + D + M (n = 10): Rats in this group received Scopolamine 20 minutes before administration of Donepezil and Mentat daily for 8 days.

On day 9, all rats were tested to see if they had retained the conditioned avoidance response. After 2 min of acclimatization period, each rat was exposed to the buzzer for 5 s. Ten such trials were given at an interval of 1 min, without giving any foot shock. Rats, responding by climbing the pole when exposed to the buzzer noise, were considered to have retained the conditioned avoidance response.

Statistical analysis

The result of the retention of CAR was analysed by the Chi-Square test. P value <0.05 was considered as significant.

RESULTS AND DISCUSSION

The percentage of rats showing retention of conditioned avoidance response (CAR) was calculated in each group. The result is shown in Table 1.

Table 1: Percentage of rats showing retention of conditioned avoidance Response

Groups	Yes (Retention of CAR %)	No (Retention of CAR %)
C	40	60
S	20	80
S+M	60	40
S+D	50	50
S+D+M	70	30

In the scopolamine administered group assessment was done by comparing the results obtained in the scopolamine pretreated (S), mentat + scopolamine (S + M) pretreated, donepezil + scopolamine (D + S) pretreated, donepezil + mentat + scopolamine (D + M + S) pretreated, and those with the control group (C). Assessment was also done by comparing mentat + scopolamine pretreated donepezil + scopolamine pretreated donepezil + mentat + scopolamine pretreated with those of the scopolamine pretreated group.

When group S is compared with group C the decrease in group S for the retention of CAR was statistically significant as compared to the group C, (where $X^2 = 8.595$ with 1 d.f. $p < 0.0001$) Scopolamine proved to be a effective model for disruption of memory.

When group S + M is compared with group S, the increase in retention of CAR in group S + M was statistically significant compared to group S, (where $X^2 = 31.688$ with 1 d.f. with $p < 0.0001$). Mentat is found to be more effective as compared to scopolamine group for retention of CAR.

When group S + M is compared with group C, there is no significant difference in retention of CAR in the two groups, (where $X^2 = 7.220$ with 1 d.f. with $p > 0.05$). However mentat treated group was numerically more significant than control group.

When group S + D is compared with group S, the increase in retention of CAR in group S + D was statistically significant compared to group S, (where $X^2 = 18.484$ with 1 d.f. with $p < 0.0001$). Donepezil is found to be more effective as compared to scopolamine group for retention of CAR.

When group S + D is compared with group C, there is no significant difference in retention of CAR in the two groups, (where $X^2 = 1.636$ with 1 d.f. with $p > 0.05$). However donepezil treated group was numerically more significant than control group.

When group S + D + M is compared with group S, the increase in retention of CAR in group S + D + M was statistically significant compared to group S, (where $X^2 = 48.505$ with 1 d.f. with $p < 0.0001$). Mentat and donepezil were more effective when used in combination and compared with scopolamine treated group.

When group S + D + M is compared with group C, the increase in retention of CAR in group S + D + M was statistically significant compared to group C, (where $X^2 = 16.990$ with 1 d.f. with $p < 0.0001$). Mentat and donepezil were more effective when used in combination and compared with control treated group.

When group S + D is compared with group S + M, there is no significant difference in retention of CAR in the two groups, (where $X^2 = 1.636$ with 1 d.f. with $p > 0.05$). However, numerically rats treated with mentat showed more retention of CAR compared to rats treated with donepezil.

When group S + M is compared with group S + M + D, there is no significant difference in retention of CAR in the two groups, (where $X^2 = 1.780$ with 1 d.f. with $p > 0.05$). However, numerically rats treated with combination of mentat and donepezil showed more retention of CAR compared to rats treated with mentat alone.

When group S + D is compared with group S + M + D, the increase in retention of CAR in group S + M + D was statistically significant compared to group S + D, (where $X^2 = 7.521$ with 1 d.f.

with $p = 0.006$), significant difference is seen. Rats treated with combination of mentat and donepezil showed more retention of CAR compared to rats treated with donepezil alone.

When the study drugs group S + M and S + D is compared with the combination group S + M + D, statistically significant difference in increase in retention of CAR in group S + M + D is seen (where $X^2 = 8.333$ with 2 d.f. with $p = 0.016$). The combination of mentat and Donepezil proved to be more effective than either of these drugs given alone. Graphical representation of comparison of the all these groups is shown in figure 1. On the X axis- distribution of groups is shown and on the Y axis – percentage of retention of CAR in each group is shown.

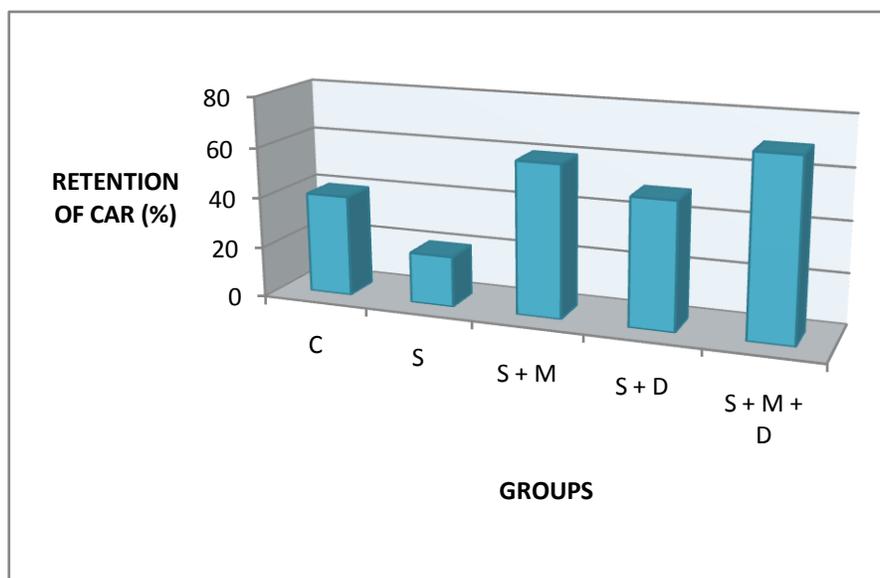


Figure 1: Graphical representation of comparison of the all the groups.

When groups of study drugs i.e. groups S + M, S + D, S + D + M is compared with the Scopolamine group (group S) the increase in the retention of CAR in groups S + M, S + D, S + D + M is highly significant ($p < 0.0001$) when compared to group S.

When the combination group of S + D + M is compared with the group S + M and S + D there is significant increase ($p = 0.016$) in the retention of CAR in group S + M + D when compared to the other two groups.

Dementia, a syndrome of many causes affects many people worldwide and can be defined as acquired deterioration in cognitive abilities that impairs the successful performance of activities of daily living. Dementia associated with probable AD is one of the most common types of dementia. Patients with AD often have cholinergic deficits in association with the disease.

Cholinergic agents either agonists or enzyme inhibitors have been put forward as therapeutic agents. Donepezil is one such candidate for a therapeutic agent in dementia, but there is evidence of some adverse effects. AchE enhances gastrointestinal motility, which may lead to diarrhoea. Central cholinesterase inhibition can lead to nausea and vomiting. Such adverse events are common to all of the cholinesterase inhibitors, however. Clinical trials have consistently shown

higher rates of gastrointestinal adverse effects in subjects treated with rivastigmine, donepezil and galantamine than in subjects receiving placebo¹⁴.

Mentat is also known as BR-16A. It contains over 20 different ingredients; the exact formulation differs between pediatric and adult presentations of the composite. Important ingredients of BR-16A, suggested to improve memory function, include the following: Jal-brahmi (*Bacopa monnieri*), Mandookaparni (*Centella asiatica*), Ashwagandha (*Withania somnifera*), Shankapushpi (*Evolvulus alsinoides*), Jatamansi (*Nardostachys jatamansi*), Vach (*Acorus calamus*), Malkangni (*Celastrus paniculatus*), and Sonth (*Zingiber officinale*). Other ingredients of BR-16A, claimed to be “nerve tonics,” include Tagar (*Valeriana wallachii*), Badam (*Prunus amygdalus*), Salap (*Orchis mascula*), Lavang (*Syzygium aromaticum*), and Pearl (*Mukta pishti*). The remaining ingredients are putative general tonics and vitalizers¹⁵.

The formulation of BR-16A is in accordance with Ayurvedic principles – different components of the formulation mutually complement each other’s properties. BR-16A is claimed in Ayurveda to enhance cognition and to ameliorate various forms of deficits in organic brain states¹⁶.

This study was designed to compare the effect of mentat and donepezil on working memory in rats using scopolamine induced memory disruption and increase in the retention of CAR as the comparative parameter. Both the drugs were well tolerated and there were no deaths during the study.

CONCLUSION

The results showed that mentat and donepezil are equally effective in preventing memory loss and there is additional benefit by combining the two drugs. Both these drugs have completely different profile regarding mechanism of action, so the combination may be useful if given for a longer period of time and this warrants further highly specified animal experiments and also needs to be evaluated in humans. The additive effect of this combination, as shown in our study, may hold great clinical significance. Addition of a cost effective drug like mentat not only lowers the overall cost of therapy but it may also improve the memory retention in neurodegenerative disorders like Alzheimer’s disease.

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