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RESEARCH ARTICLE

Modulation of working memory by Mentat and Donepezil using ECT induced amnesia in rats.

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ABSTRACT:
Objective: To compare the effect of Mentat with Donepezil on working memory in rats. Materials and Methods: The rats were trained for conditioned avoidance response (CAR) by using Cook's pole climbing apparatus. Rats trained for CAR received a single maximal electroconvulsive shock (150V, 50 Hz sinusoidal with intensity of 210 mA) for 0.5 sec duration through crocodile clip ear electrodes from a small animal electroconvulsiometer without any anaesthesia. During the study, a single ECS was administered daily for 8 consecutive days. This is an established model known to produce amnesia which was used to evaluate the effect on learning and memory of the 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 analyzed by the chi-square test. Results: Findings show that administration of single ECS daily for 8 days before the test run on the apparatus causes transient amnesia resulting in disruption of retention of CAR. There was no significant difference in the retention of CAR between the groups receiving Mentat and Donepezil. Also, retention of CAR in the group that received combination of these two drugs was not significant when compared to the groups receiving individual drug. Conclusion: Mentat with memory retention capacity similar to Donepezil may be used as an alternative in the treatment of dementia. Results from this study provide a good platform to conduct clinical studies to assess the benefit of Mentat in Alzheimer's disease.
Keywords: Working memory, Mentat, Donepezil, Electroconvulsive shock, Conditioned avoidance response.

INTRODUCTION

Memory, one of the complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay. We have sensory memory lasting for a few seconds, short-term memory, lasting for few hours and long term memory, where in the information is stored for several years or even life time[1].

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 areas[2]. 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. 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 behavior and signs of cortical disconnection including apraxia, aphasia and agnosia[5]. 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 as $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 patient[6].

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 institutionalization 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 inhibitors[7].

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 damage[8]. Ayurvedic formulations are generally multicomponent and are based on the concept that such combinations provide synergistic therapeutic effects and eliminate any adverse reactions (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 Electroconvulsive Therapy (ECT) to produce amnesia.

MATERIALS AND METHODS

Animals

Experimentally naïve Sprague Dawley albino rats weighing between 150 and 200g of either sex were used. The rats were maintained under standard conditions of temperature ($25^{o}C\pm5^{o}C$), relative humidity (55±10%) and a 12/12 h 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

Electroconvulsiometer 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 Himalaya Drug Company, Bengaluru, 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 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 10 trials were considered to have developed conditioned avoidance response for further experiments[11].

Mentat Tablets Composition:

Contains extracts of the following herbs

1. Brahmi - Bacopa monnieri 136mg
2. Mandukaparni - Centella asiatica 70 mg
3. Ashwagandha - Withania somnifera 52mg
4. Vishnukranthi - Evolvulus alsinoides 52 mg
5. Jatamansi - Nardostachys jatamansi 52mg
6. Tagar - Valeriana wallichii 50 mg
7. Vaividang - Embelia ribes 50mg
8. Vatima - Prunus amygdalus 50mg
9. Vacha - Acorus calamus 42mg
10. Amalaki - Emblica officinalis 36mg
11. Haritaki - Terminalia chebula 36mg
12. Guduchi - Tinospora cordifolia 36mg
13. Jyotishmati - Celastrus paniculatis 32mg
14. Shyonaka - Oroxylum indicum 32 mg

Contains powder of the following herbs

1. Brahmi - Bacopa monnieri 80mg
2. Kapikachchu - Mucuna pruriens 18mg
3. Green Cardamom - Elettaria cardamomum 18mg
4. Arjuna - Terminalia arjuna 18mg
5. Fennel - Foeniculum vulgare 18 mg
6. Vidari kanda - Ipomoea digitata 18 mg
7. Salabmisri - Orchis mascula 18,g
8. Dried Ginger - Zingiber officinale 14mg
9. Bibhitaki - Terminalia bellirica 14 mg
10. Jaiphal - Myristica Fragrans 14 mg
11. Clove - Syzgium aromaticum 10mg
12. Mukta Pishti 3mg

ECT induced disruption of memory

The rats were given a single maximal electroconvulsive shock (150V, 50 Hz sinusoidal with intensity of 210 mA) for 0.5 sec duration through crocodile clip ear electrodes from a small animal electroconvulsiometer without any anaesthesia. During the study, a single ECS was administered daily for 8 consecutive days[12].

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 response[13].

III. Double distilled water was used as a vehicle for dissolving both the study drugs and 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 intraperitoneal route or oral group depending on the group. The rats were divided into following groups:

Control (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.

ECS (E)(n=10): Rats in this group received ECS at 150V, 50 cps, 0.5 sec. through pinnal electrodes daily for 8 days. This group showed the effect of ECS on working memory in rats.

E + Mentat (M)(n=10): In this group rats received ECS as mentioned above and Mentat in a dose of 200mg/kg orally for 8 days.

E + Donepezil (D)(n=10): will receive ECS and Donepezil in a dose of 0.32 mg/kg by intraperitoneal route.

E + D + M (n=10): In this group rats receive ECS, Donepezil and Mentat in above mentioned doses for 8 days. This group was used to study the modulation of effects of the study drugs on the working memory of rats by combination.

All the cages were tagged as per the groups mentioned above. All the rats were confined to their respective cages.

On day 9, all rats were tested to see if they retained the conditioned avoidance response. After 2 min of acclimatization period, each rat was exposed to the buzzer for 5s. 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 analyzed by the Chi-Square test. P value < 0.05 was considered significant.

RESULTS

The percentage of rats showing retention of conditioned avoidance response (CAR) was calculated in each group. The results are 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**
E	**20**	**80**
E+M	**80**	**20**
E+D	**70**	**30**
E+D+M	**80**	**20**

When group E is compared with group C the decrease in group E for the retention of CAR was statistically significant as compared to group C. (where $\varkappa^2$ = 8.595 with 1 d.f $p<0.0001$, Highly significant difference is seen).

When group E+ M is compared with group E, the increase in retention of CAR in group E+ M was statistically significant compared to group E. (where $\varkappa^2$ = 69.620 with 1 d.f with $p< 0.0001$, Highly significant difference is seen here).

When group E+M is compared with group C, the increase in retention of CAR in group E + M was statistically significant compared to group C.(where $\varkappa^2$ = 31.688 with 1 d.f with $p<0.0001$, Highly significant result is seen here).

When group E+D is compared with group E, the increase in retention of CAR in group E + D was statistically significant compared to group C.(where $\varkappa^2$ = 48.505 with 1 d.f with $p<0.0001$, Highly significant result is seen here).

When group E+D is compared with group C, the increase in retention of CAR in group E + D was statistically significant compared to group C.(where $\varkappa^2$ = 16.990 with 1 d.f with $p<0.0001$, Highly significant result is seen here).

When group E+D+M is compared with group E, the increase in retention of CAR in group E + D + M was statistically significant compared to group E.(where $\varkappa^2$ = 69.620 with 1 d.f with $p<0.0001$, Highly significant result is seen here).

When group E+D+M is compared with group C, the increase in retention of CAR in group E + D + M was statistically significant compared to group C.(where $\varkappa^2$ = 31.688 with 1 d.f with $p<0.0001$, Highly significant result is seen here).

When group E+D is compared with group E+M, there is no significant difference in the retention of CAR between the two groups. (where $\varkappa^2$ = 2.160 with 1 d.f with $p>0.05$, No significant difference is seen here).

When the groups E+D and E+M are compared with group E+M+D, there is no significant difference in the retention of CAR in the three groups. (where $\varkappa^2$ = 3.727 with 2 d.f with p>0.05, No significant difference is seen here).

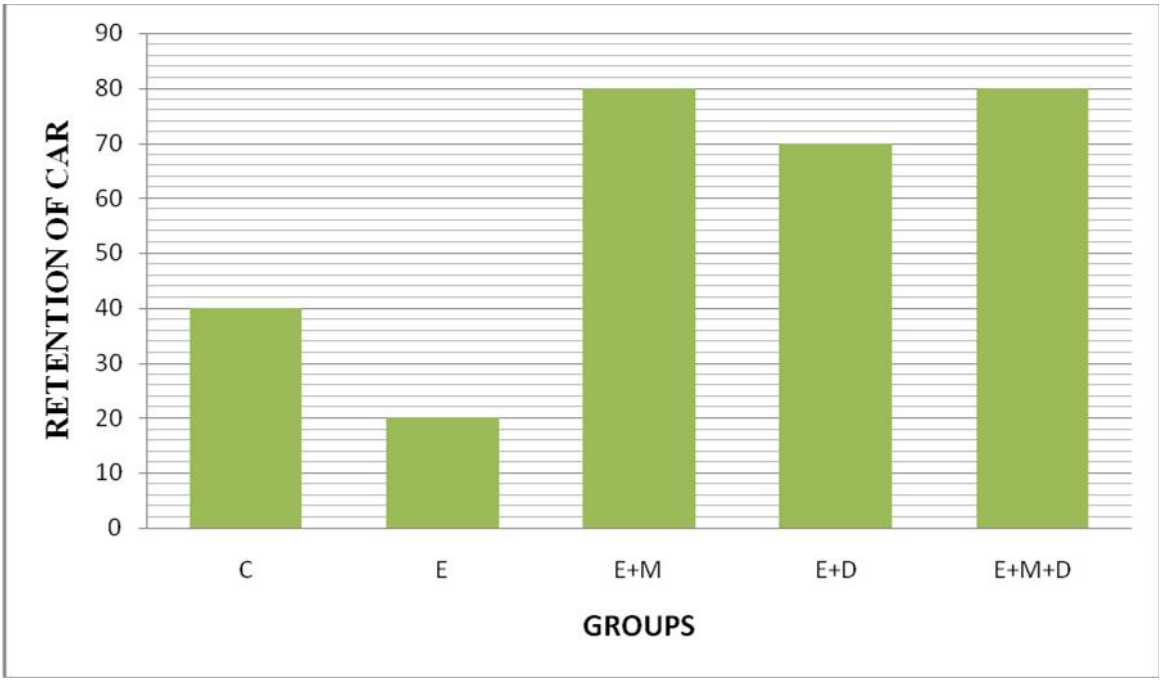

Fig.1 Comparison of retention of CAR in various groups

DISCUSSION

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. Cholinesterase inhibitors enhance gastrointestinal motility, which may lead to diarrhea. 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 placebo[14].

Mentat 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 vitalizers[15].

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

This study was designed to compare the effect of Mentat and Donepezil on working memory in rats using electroconvulsive therapy 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 there was no difference between Mentat and Donepezil in preventing memory loss. The combination of these two drugs did not prove to be beneficial as compared to using Donepezil or Mentat alone. Both these drugs have completely different profile regarding mechanism of action and this warrants further highly specified animal experiments and also needs to be evaluated in humans. The lack of an additive effect of this combination, as shown in our study, may disapprove the use of Mentat as an add-on drug. Instead with memory retention capapcity similar to Donepezil, a cost effective drug like Mentat may actually serve as an alternative to existing therapy in neurodegenerative disorders like Alzheimer's disease.

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