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Memory Enhancing Activity of Saraswatarishta in Mice

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a gradual decline in memory. Incidence of Alzheimer's disease increases with age. The disease incidence is 1% in 60 year olds & increases to 30 % at 85 years age. Hence this disease is already having enormous magnitude in today's graying world. Current treatment of Alzheimer's disease includes- cholinesterase inhibitors & N-methyl- D-aspartate antagonists, but the benefit observed is modest. In traditional medicine Saraswatarishta is being used as memory enhancer for centuries. Brahmi, one of its major ingredients, is also being used to treat Alzheimer's. So present study was undertaken for authentication of traditional claims of Saraswatarishta as a memory-enhancing agent. Five groups of mice (6 mice in each) were used for this study. Control group (group I) received distilled water, Group II received Saraswatarishta (2.5ml/kg) single dose and Group III received Saraswatarishta (2.5ml/kg) for 2wks. Group IV was given Diazepam (1mg/kg) to produce amnesia. For Group V, Saraswatarishta (2.5ml/kg) was given for 2wks followed by Diazepam (1mg/kg). Effect of Saraswatarishta on learning and memory of mice was studied using elevated plus maze model (EPM). Reduction in TL (Transfer Latency) indicates improvement in learning or memory and prolongation indicates impairment. Diazepam induced prolongation of TL is an accepted model of dementia. In our study, 2 weeks daily treatment of Saraswatarishta completely prevented impairment of learning and memory by Diazepam, corroborating the Ayurvdic use of Saraswatarishta and Brahmi, its major ingredient in the management of dementia. Saraswatarishta can be used as preventive measure to overcome demensia in Alzheimer's disease.

Keywords: Alzheimer's disease, Dementia, Learning, Memory, Saraswatarishta.

'Population ageing' has become a key word in this century. One of the problems which has gained prominence with increased longevity is Alzheimer's disease - a progressive neurodegenerative disorder characterized by a gradual decline in memory.

Asia is already said to have greater prevalence of Alzheimer's disease than other continents.[1]It is further predicted to triple in the Asia pacific region by 2050. This will constitute more than half of total number of people suffering from dementia in the world.[2]

Memory enhancers find an important application in patients of dementia including Alzheimer's.[3]These drugs are also being increasingly promoted and used for better performance even by healthy people faced with increasing demands of the competitive environment.

Saraswatarishta is one such well-known Ayurvedic polyherbal formulation traditionally being used as a brain tonic to improve memory. It is also used to treat neurosis, psychosis, insomnia, epilepsy,stammering and memory loss.[4]Brahmi, the main ingredient of Saraswatarishta, is used for revitalization of brain and nervous system. It is also used for nourishing intellectual clarity and sharpness.[5]

However, there is very little scientific data corroborating the effect of Saraswatarishta on learning and memory in healthy and diseased persons.The present study was therefore undertaken for authentication of traditional claims of Saraswatarishta as a memory enhancer, using an animal model.

MATERIALS AND METHODS

Animals

Albino mice of either sex were obtained from the Central Animal house of our Institute with CPCSEA (Committee for the purpose of control and supervision of experiments on animals) approval. Study was conducted after protocol approval from Institutional Animal Ethics Committee (IAEC). Care of animals was taken as per the guidelines of CPCSEA.

Thirty mice weighing around 19-21 grams (age approximately 1 month) were used for this study. All the animals were acclimatized to the laboratory conditions for 5 days before the study. The animals had free access to food and water. Alternate light and dark cycles of 12 hrs. were maintained. All experiments were carried out during daytime from 10.00 a.m. to 2.00 p.m.

Apparatus

Elevated plus- maze

The elevated plus- maze was used as described by Milind Parle et a1.[1] The technique, end point and criteria for testing learning and memory were as per the parameters described by the investigators in the area of neuropsychopharmacology.[1,6,7,8]The elevated plus-maze consists of two open arms (16cm x 5 cm) intersecting with two closed arms (16cm x 5cm x 12cm) at right angles with a central platform (5cm x 5cm) at the intersection. The entire maze is at a height of 25 cm from the floor.

Transfer Latency (TL) was the parameter used for assessing learning and memory with this model. For assessing the TL, each mouse was placed at the end of an open arm, facing away from the central platform. TL was measured as the time taken by the mouse to move into one of the closed arms with all its four legs. The mouse was allowed to explore the maze for another 10 seconds. Cut off time given for entering the closed arm was 90 seconds. Animals which fail to enter the closed arm within 90 seconds or those which fall off the maze were excluded from trial. Care was taken to keep the maze clean while taking observations.

TL of the first exposure to the elevated plus maze (observation 1) indicates learning. Second observation of TL (observation 2), recorded after 24 hours of first exposure (observation 1) indicates retention of learning or memory. Reduction in TL in the second observation indicates improvement in learning or memory and prolongation indicates impairment.

Significant increase in Transfer Latency at first exposure to the Elevated Plus Maze by a single dose of Diazepam was considered to indicate significant impairment of learning, while significantly greater TL2 measured 24 hrs. later was indicative of impaired memory. This impairment of learning and memory by Diazepam on Elevated Plus Maze is recognized model for testing drugs

Table 1. Treatment Groups

Group (n=6)	Drug Treatment	Dose
I (Control)	Distilled water(control)	10 ml/kg (oral)
II (SR SD)	Saraswatarishta (Single Dose)	2.5 ml/kg (oral)
III (SR 2wks)	Saraswatarishta(2wks)	2.5 ml/kg/day (oral)
IV (Diazepam SD)	Diazepam (SD)	1mg/kg(I.P.)
V (SR+Diazepam)	Saraswatarishta (2wks)+ Diazepam SD on day 14	2.5ml/kg/day (oral) + 1mg/kg (I.P.)

SR-Saraswatarishta, SD – Single Dose,I.P.-Intraperitonial

effective in treatment of conditions associated with Dementia like Alzheimer's disease. [9,10,11]

Drugs

1. Saraswatarishta: Commercially available Saraswatarishta of Baidyanath Pharmaceuticals was used for this study. Oral dose of Saraswatarishta in mice was obtained by computing from the human dose for memory enhancement, was 2.5ml/kg.[12]

2. Inj. Diazepam from Ranbaxy was used in this study, diluted in normal saline. Diazepam was given intraperitonially in the dose of 1mg /kg.

Saraswatarishta Ingredients: 7 major and 16 minor ingredients[13]

1. Major ingredients: 'Brahmi'- a MedhaRasayana, Shatavari, Vidarikand, Harada, Khas, Adrak, Saunf.

2. Minor ingredients: Shahad, Chini, DhayPhool, Renuka, Nishoth, Chotpeepal, Laung, Bach, Kooth, Asaghendh, Beheda, Giloy, ChotiElaichi, Vayvirang, Swaranapatra, Dalchini

Drug protocol

Albino mice were divided into 5 groups of 6 animals each.

Group I was given distilled water orally (10 ml /kg) which served as vehicle control. TL was noted 45 min after oral administration of water (Observation 1) and again after 24 hrs. (Observation 2).

Group II was given Saraswatarishta (2.5 ml / kg) single dose (SD). TL was noted 45 min. after drug administration (observation 1) and again after 24 hrs. (Observation 2).

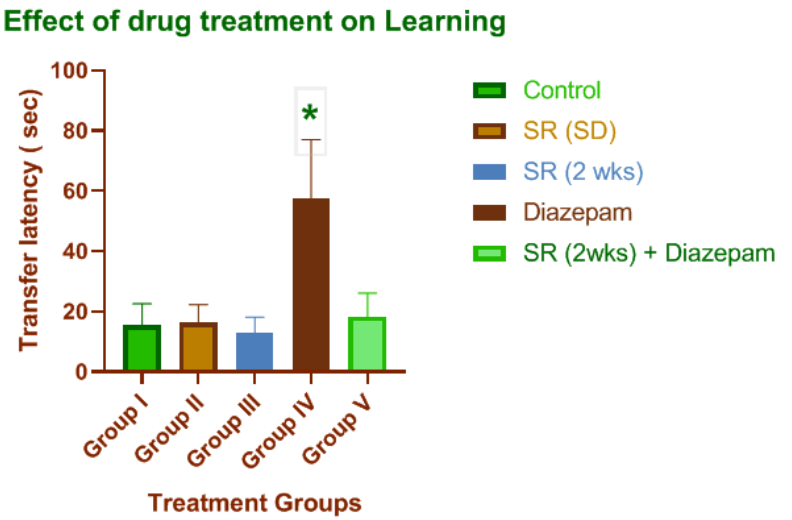
Group III was given Saraswatarishta (2.5 ml /kg/day) for 2 weeks. TL was noted 45 min after the last dose i.e. on day 14 (observation 1) and again after 24 hrs. (Observation 2).

Group IV was given Diazepam (1 mg /kg) single dose I.P. TL was noted 45 min after diazepam injection (observation 1) and again after 24 hrs. (Observation 2).

Group V was given Saraswatarishta (2.5ml /kg /day) for 2 weeks and Diazepam was injected I.P (1mg /kg), 60 min after administration of last dose of Saraswatarishta on day 14. TL was noted 45 mins after administration of Diazepam (observation 1) and again 24 hrs. later i.e., on day 15 (observation 2).

RESULTS

TL values were recorded in seconds which were expressed as Mean ± SEM for each group. Results were analysed by Kruskal Wallis test followed by Mann Whitney test to compare pairs of groups.



Graph 1

Caption: TL measured at first exposure to the Elevated Plus Maze 45 minutes after the drug treatment (TL 1) in the 5 groups.

SR-Saraswatarishta, SD-Single Dose

* indicates significant difference from all other groups

TL values of group II& III were compared with those of Group I to see effect of Saraswatarishta on learning and memory.

TL values of Group IV were compared with those of group I to see effect of Diazepam on learning and memory.

TL values of group V were compared with group IV to see the preventive effect of Saraswatarishta on Diazepam induced impairment in Learning and memory.

Effect on Learning

Graph 1 shows comparison of TL on first exposure to the maze 45 minutes after drug treatment (TL 1), which represents learning ability of animals. Comparing these TL 1 values of all groups by Kruskal Wallis test indicated that the learning ability was not same in all the groups.

Applying Mann Whitney test to pairs of groups revealed that Saraswatarishta treated Groups II, III and V did not differ significantly from each other or from control Group I.

No difference between TL 1 of Saraswatarishta treated Groups II, III and control Group I indicates that Saraswatarishta itself has no effect on learning, either beneficial or adverse.

TL 1of Diazepam treated Group IV was significantly greater than that of control as well as that of Saraswatarishta treated Groups II, III and V

Significantly greater TL 1 of Diazepam treated Group IV compared to control indicates significant impairment of learning by a single dose of Diazepam.

Significantly less TL 1 of Group V (2 weeks pretreatment with Saraswatarishta before Diazepam) than Group IV (Diazepam alone) indicates that 2 weeks Saraswatarishta pretreatment afforded significant protection in mice from impairment of learning due to Diazepam.

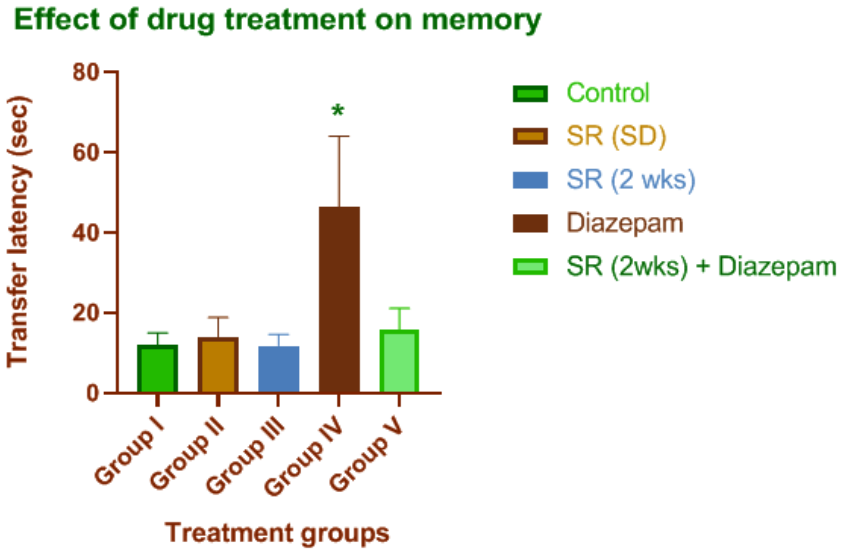
Absence of difference between Group V and control group indicates complete protection by Saraswatarishta.

Effect on memory

Graph 2 shows TL noted 24 hours after 1st exposure (TL 2), indicative of memory.

Comparing TL 2 values of all groups by Kruskal Wallis test indicated that the groups were not homogeneous as far as effect on memory was concerned.

Applying Mann Whitney test to pairs of groups revealed that Saraswatarishta treated Groups II, III and V did not differ significantly from each other or from control Group I. This indicates that this treatment with Saraswatarishta by itself had no effect on memory.



Graph 2

Caption: TL measured 24 hours later the drug treatment (TL 2) in the 5 groups.
SR-Saraswatarishta, SD-Single Dose
* indicates significant difference from all other groups

TL 2 in Diazepam treated animals (Group IV) was significantly greater than control group (Group I) as well as all Saraswatarishta treated groups.

Significantly increased TL 2 in Diazepam treated animals (Group IV) as compared to control group indicates that single dose of Diazepam significantly impaired memory.

Significantly less TL 2of Group V (2 weeks pretreatment with Saraswatarishta before Diazepam) than Group IV (Diazepam alone) indicates that 2 weeks Saraswatarishta pretreatment afforded significant protection in mice.

No significant difference between Group V and control indicates complete protection, nullifying the impairment of memory due to Diazepam.

DISCUSSION

Our study showed that 2 weeks pretreatment with Saraswatarishta offered complete protection against impairment of learning as well memory caused by Diazepam. However, Saraswatarishta treatment by itself has shown no effect on learning or memory, either beneficial or adverse.

Protection against dementia

Prolongation of Transfer latency on the Elevated Plus maze by Diazepam is a recognized model for testing drugs effective in treatment of conditions associated with Dementia like Alzheimer's disease.[9,10,11]Two weeks pretreatment with Saraswatarishta in our study has completely blocked this effect of Diazepam. Uma et al, have also observed protection against diazepam induced amnesia in mice with just 7 days of pretreatment with Saraswatarishta.[5]

In Ayurveda, disorders related to memory like Dementia, Alzheimer's disease are considered as 'Vata vyadhi'. 'Medhyarasayanas' like 'Brahmi', 'Guduchi', 'Haritaki', and 'Shatawari' are used in the treatment of such disorders.[13,14]

Saraswatarishta is rich in Medhyarasayan Brahmi. Beneficial effect of Saraswatarishta in our study corroborates this therapeutic use of Brahmi. Saraswatarishta itself can also be used to prevent dementia associated with ageing.

According to Ayurveda, Saraswatarishta has potential to increase Tej, Bal, Oaj, Veerya, Medha and Smruti (Strength, Intellect, Memory). It is claimed to control stress and improve work efficiency. It is specifically effective in improving memory impaired by any cause like old age, trauma, injury, infections.[13]

Different authors have also postulated mechanism for this beneficial effect of Saraswatarishta. The protection offered by Saraswatarishta may be due to increased synthesis of acetylcholine in the brain[15] or by supplementation of this central action of choline by Tinospara Cordifolia, an important ingredient of Saraswatarishta.[16] Brahmi, the major ingredient of Saraswatarishta, is also postulated to exert beneficial effects by preventing destruction of Acetylcholine, reduction in beta amyloid and giving neuroprotective action by antioxidant effect and increased cerebral blood flow[17].

Alzheimer's disease (AD) accounts for about 70% of all cases of dementia in the elderly, placing an enormous burden on the patient's family and the society. Hence efforts are on, on war footing, to develop agents effective against Alzheimer's disease and other cognitive disorders.[18] Currently available treatment options for AD in allopathy include cholinesterase inhibitors Tacrine, Donepezil and Rivastigmine[19] and N-methyl-D-aspartate antagonist Memantine.[20]

But their benefits have been found to be modest, particularly in terms of limited duration of benefit. A lot of individual variation is also seen in the beneficial effects.[21] In clinical trials, cholinesterase inhibitors have been shown to benefit fewer than 50% of patients.[17]Antioxidants like vit. E and C are also being prescribed to counter the harmful effects of free radicals thought to be responsible for the problems of ageing, including dementia.[1,22] Further expansion of this drug armamentarium faces the challenge of difficulty in recruiting participants, inadequate knowledge about pathophysiology of the disease condition and prolonged duration required to assess the beneficial effects.[21]

In the face of these difficulties, it would be a great asset if we could validate the effectiveness of Ayurvedic Medicines proclaimed to have benefits in these conditions. Use of traditional Indian medicines like Saraswatarishta along

with allopathic medicines may also increase the therapeutic benefits of the allopathic treatment.

Enhancement of learning and memory

Ayurvedic formulation Saraswatarishta is also recommended as a memory enhancer and continues to be used to boost learning and memory in this competitive world. However, in our study, single dose or even 2 weeks of Saraswatarishta treatment by itself showed no effect on learning and memory in mice. Nor did we find any other studies assessing effect of Saraswatarishta on learning and memory in animals. However, Antidepressant effect of Saraswatarishta in animal model of behaviour despair has been proven by Parekar RR et. al.[23]

We did come across reports of assessment of effects of Brahmi, the major ingredient of Saraswatarishta, on learning and memory, both in man and animals. Beneficial effects of Brahmi ghrita on learning and memory have been reported by G. Achliya et al, in experimental animals[24]. On the other hand, as in our study, a review of six human studies involving adults with little or no cognitive impairment did not show any enhancement of cognitive domain after 12 Wks. of treatment with various extracts of Brahmi.[25]

CONCLUSION

Thus, Saraswatarishta pre-treatment for 2 wks. offered protection to animals against impairment of learning and memory by Diazepam. According to this observation it can be used as a preventive measure to overcome dementia in Alzheimer's disease. Saraswatarishta single dose or chronic treatment for 2 weeks did not show any beneficial effect on learning or memory. To confirm beneficial effects of Saraswatarishta in demensia, further studies are planned to see its effects on patients of Alzheimer's disease.

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Conflict of Interest

None

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None

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