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EFFECT OF SARASWATARISHTA ON LEARNING AND MEMORY

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ABSTRACT:

Dementia is characterized by multiple cognitive defects that including Alzheimer's disease, leads to a progressive loss of mental functions. The present study was aimed at investigating the effects of Saraswatarishta, an ayurvedic polyherbal formulation on memory in swiss albino mice. The drug Saraswatarishta was administered orally in two doses (1ml/kg and 2ml/kg). Amnesia was induced in mice by intraperitoneal injection of diazepam (1mg/kg). Elevated plus-maze and Hebb's William maze apparatus served as the exteroceptive behavioral models for memory. Saraswatarishta (1ml/kg and 2ml/kg, p.o) produced a dose-dependent improvement in learning and memory in mice. Furthermore, it reversed the amnesia induced by diazepam (1mg/kg, i.p). It may prove to be a useful remedy for the management of Alzheimer's disease.

Key words: Saraswatarishta, Ayurveda, Memory, Alzheimer's disease, Dementia.

INTRODUCTION:

Alzheimer's disease is a chronic, progressive and disabling organic brain disorder characterized by disturbance of multiple cortical functions including memory, judgement, orientation, comprehension, learning capacity and language. (Jay and Ellis, 2005).Reducing oxidative stress by anti-oxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and increasing the synthesis of acetycholine which improves learning and memory by the use of immunostimulants are some positive approaches to management for Alzheimer's disease (Wood, 2004).Ayurvedic drugs have been shown to successfully attenuate memory dysfunctions induced dy diazepam (Hanumanthachar and Milind, 2006).

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The current study was aimed to investigate the effects of Saraswatarishta, an Indian Ayurvedic poly-herbal formulation on memory in mice. It was found as used as an appetizer, anti-anxiety and a rejuvenator. Each 100ml of the Saraswatarishta contains *Bacopa monnieri* (Brahmi) 23.5g, *Asparagus racemosus* (Shatavari) 5.8g, *Pueraria tuberose* (Vidari) 5.8g, *Terminalia chebula* (Haritaki) 5.8g, *Vetivera zizanoides* (Ushira) 5.8g, *Zingiber officinale* (Shunthi) 5.8g, *Foeniculum vulgare* (Mishreya) 5.8g, *Woodfordia fructosa* (Dhataki) 5.8g, *Piper aurantiacum* (Renuka) 0.294g, *Operculina turpethum* (Trivrit) 0.294g, *Piper longum* (Pippali) 0.294g, *Acorus calamus* (Vacha) 0.294g, *Saussurea lappa* (Kushtha) 0.294g, *Withania somnifera* (Ashwagandha) 0.294g, *Embelia ribes* (Vidanga) 0.294g, *Tinospora cordifolia* (Guduchi) 0.294g, *Terminalia belerica* (Bibhitaka) 0.294g, *Cinnamomum zeylanicum* (Tavk) 0.294g, *Elettaria cardamomum* (Sukshmaila) 0.294g, Syzygium aromaticum (Lavanga) 0.294g, Madhu 11.7g, Sharkara 29g and water Q.S

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MATERIALS AND METHODS:
Animals: Swiss albino mice of both sex weighing around 25-30gm were selected in the present study. Animals were procured from our animal house. They were acclimatized to the laboratory conditions for 5 days before doing the experiment. The animals were provided with alternate light and dark cycles of 12 hours each. All experiment was carried out during daytime from 9 and 18 hours.

Drugs: The drugs used in this study were obtained from local markets. Diazepam (Calmpose, Ranbaxy, India), Piracetam (Nootropil, UCB India Ltd, India), saraswatarishta (The Indian Medical Practitioner's Co-operative Pharmacy and Stores Ltd, Chennai).

Methods:
a) Elevated plus-maze : The elevated plus maze for mice consists of two open arms (16×5 cm^2) and two covered arms ($16 \times 5 \times 12$ cm^2) extended from a central platform (5×5cm^2) and the maze was elevated to a height of 25 cm from the floor. On the first day (i.e. seventh day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24hours after last dose (Dhingra and Parle, 2003).
b) Hebb-William maze: The Hebb-William maze is also called as rectangular maze. The maze consists of completely enclosed rectangular box with an entry and a reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just one twisting corridor leading from the entry to the reward chamber.

The learning assessment for control and drug treated mice was conducted at end of treatment under zero watt red coloured bulb so as to minimize the nocturnal cycle disturbances. On the 16th day all the mice were familiarized with Hebb-William maze for a period of 10 min. From17th -20thday, the mice received four consecutive trials of training per day in the maze. In each trial the rat was placed in the entry chamber and the timer was activated as soon as the rat leaves the chamber. The time taken for the mice to reach the reward chamber (TRC) was taken as the learning score of the trial. The average of four trials was taken as the learning score for the day. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. During learning assessment, the animals were

exposed to food and water *ad libitum* only for 1 h after the maze exposure for the day was completed to ensure motivation towards reward area (Ashutosh Agarwal *et al.*, 2002).

Experimental protocol:
a) Elevated plus-maze: In the present study the animals were divided into 5 groups. Each group comprised of a 5 animals.

Group-I served as the Control. Transfer latency was tested on the 7th day and after 24 hours (i.e on 8th day) by using elevated plus-maze.

Group-II treated with Diazepam (1mg/kg) was injected i.p on the 7th day and retention was measured after 24 hours (on 8th day) by using elevated plus-maze.

Group-III treated with Piracetam (400mg/kg, i.p) was used as a nootropic agent and was injected for 7 days. After 60 minutes of the administration of the last dose (i.e on 7th day), the amnesia inducing agent Diazepam (1mg/kg) was injected i.p. The animals were exposed to the training session after 45 minutes and the retention was measured after 24 hours by using elevated plus maze.

Group-IV treated with Saraswatarishta (1ml/kg) orally for seven successive days. After 60 minutes of the administration of the last dose (i.e. on 7th day), the amnesia inducing agent Diazepam (1mg/kg) was injected i.p. The animals were exposed to the training session after 45 minutes and the retention was measured after 24 hours by using elevated plus maze.

Group-V treated with Saraswatarishta (2ml/kg) orally for seven successive days. After 60 minutes of the administration of the last dose (i.e. on 7th day), the amnesia inducing agent Diazepam (1mg/kg) was injected i.p. The animals were exposed to the training session after 45 minutes and the retention was measured after 24 hours by using elevated plus maze.

b) Hebb-William maze: In the present study the animals were divided into5 groups. Each group comprised of a 5 animals.

Group-I served as the control.TRC was tested by using Hebb-William maze.

Group-II treated with Diazepam (1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze.

Group-III treated with Piracetam (400mg/kg, i.p) for 15 days. The amnesia inducing agent Diazepam (1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze.

Group-IV treated with Saraswatarishta (1ml/kg) orally for 15 days. The amnesia inducing agent Diazepam (1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze.

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Group-V treated with Saraswatarishta (2ml/kg) orally for 15 days. The amnesia inducing agent Diazepam (1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze.

Statistical Analysis: The values are expressed as mean±SEM. The results were analyzed for statistical significance using student's t test.

RESULTS:

a) Elevated plus-maze apparatus: Diazepam (1mg/kg) treated animals showed higher transfer latency values (time in seconds) on the 7th day and after 24 hours (i.e. 8th day) as compared to control group, indicating impairment in learning and memory.

Piracetam (400mg/kg) treatment for 7days decreased transfer latency values (time in seconds) on the 7th day and after 24 hours (i.e. 8th day) as compared to the control group, indicating that piracetam (400mg/kg) pre-treatment for 7 days reversed impairment in learning and memory caused by diazepam.

Saraswatarishta (1ml/kg) treatment for 7 days decreased transfer latency (P< 0.5) on the 7th day and (P<0.01) after 24 hours (i.e.8th day) as compared to the control groups.

Saraswatarishta (1ml/kg) treatment for 7 days decreased transfer latency (P< 0.1) on the 7th day and (P<0.001) after 24 hours (i.e.8th day) as compared to the Diazepam groups. It indicates that the saraswatarishta

(1ml/kg) pre-treatment for 7 days reversed impairment in learning and memory caused by diazepam.

Saraswatarishta (2ml/kg) treatment for 7 days showed marked decrease in transfer latency values (P< 0.01) on the 7th day and (P< 0.01) after 24 hours (i.e. 8th day) as compared to the control groups.

Saraswatarishta (2ml/kg) treatment for 7 days showed marked decrease in transfer latency values (P< 0.001) on the 7th day and (P< 0.001) after 24 hours (i.e. 8th day) as compared to the Diazepam groups. It indicates that the saraswatarishta (2ml/kg) pre-treatment for 7 days reversed impairment in learning and memory caused by diazepam.

b) Hebb-William maze apparatus: The time taken by the animal (Learning score) to reach the reward chamber (B) from the entry chamber (A) in saraswatarishta 1ml/kg treated animals was reduced from 78.3±14.4, 117.8±10.1, 96.2±10.9, 83.4±8.49 seconds (Diazepam control) to 69.9±9.84, 44.5±3.09, 39.2±4.22, 44.9±2.23 seconds on day1,2,3 and 4 respectively.

The time taken by the animal (Learning score) to reach the reward chamber (B) from the entry chamber (A) in saraswatarishta 2ml/kg treated animals was reduced from 78.3±14.4, 117.8±10.1, 96.2±10.9, 83.4±8.49 seconds (Diazepam control) to 44.4 ±2.46, 46.2±4.67, 39.4±4.87, 51.8±4.92 seconds on day 1,2,3 and 4 respectively. All the learning scores were comparable to that of piracetam treated animals.

Table1: Effect of Saraswatarishta on transfer latency by using Elevated Plus Maze

Groups n=5	Dose	Transfer latency on last day treatment (in seconds)	Transfer Latency After 24 Hours(in seconds)
Control	_	100±8.37	96±3.99
Diazepam	1mg/kg	109±3.87	111±3.79
Piracetam + Diazepam	400mg/kg + 1mg/kg	73±13.48	59±4.85
Saraswatarishta + Diazepam	1ml/kg + 1mg/kg	72±18.28$_a$*	60±9.09$_c$[#]
Saraswatarishta + Diazepam	2ml/kg + 1mg/kg	68.75±2.27$_b$**	42.5±10.41$_c$[#]

* P<0.5, **P<0.01 Vs control (last day treatment). [#] P<0.01 Vs control (after 24 hours).a-P<0.1, b-P<0.001 Vs diazepam (last day treatment).c- P< 0.001 Vs diazepam (after 24 hours).

Table 2: Effect of Saraswatarishta on learning scores of mice on day 1-4 in Hebb-William Maze Apparatus.

Groups n=5	Dose	Learning scores (time in seconds)			
		Day-1	Day-2	Day-3	Day-4
Control	_	58.9±2.15	33.2±4.16	46.6±4.61	45.4±8.04
Diazepam	1 mg/kg	78.3±14.4	117.8±10.1	96.2±10.9	83.4±8.49
Piracetam + Diazepam	400 mg/kg + 1mg/kg	72.7±12.4	67.5±4.51	54.8±6.04	60.6±6.01
Saraswatarishta + Diazepam	1 ml/kg + 1mg/kg	$69.9 \pm 9.84_a$*	$44.5 \pm 3.09_a$#	$39.2 \pm 4.22_b$*	$44.9 \pm 2.23_b$*
Saraswatarishta + Diazepam	2 ml/kg + 1mg/kg	$44.4 \pm 2.46_c$**	$46.2 \pm 4.67_a$#	$39.4 \pm 4.87_b$*	$51.8 \pm 4.92_d$*

* $P<0.5$, # $P<0.1$, ** $P<0.01$ Vs control. a-$P<0.001$, b-$P<0.01$, c-$P<0.05$, d-$P<0.02$ Vs diazepam groups.

DISCUSSION:

The clinical features of Alzheimer's disease are an amnesic type of memory impairment, deterioration of language, motor and sensory abnormalities (Wood, 2004). Inspite of the high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, we were motivated to explore the new approach in Indian traditional system to manage Alzheimer's disease.

Bacopa monnieri, Withania somnifera, Tinospora cordifolia, Terminalia chebula (Inamdar and Rajarama Rao, 1962) *Foeniculum vulgare, Acorus calamus, Zingiber officinale, Syzygium aromaticum, Piper longum* and *Piper aurantiacum* (Nadkarni, 2002) were proved to possess memory enhancing effects that present in Saraswatarishta may involve in the reversal of memory deficit in this present investigation.

Immunohistochemical studies suggested that existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that the non-steroidal anti-inflammatory drug Indomethacin halted the progressive memory loss seen in Alzheimer's disease patients (McGeer, 1999). The constituents of Saraswatarishta such as *Vetivera zizanoides, Pueraria tuberose, Operculina turpethum, Acorus calamus, Withania somnifera, Bacopa monnieri, Tinospora cordifolia, Asparagus racemosus, Foeniculum vulgare* (Choi and Hwang)*, Piper longum, Elettaria cardamomum* and *Saussurea lappa* (Majumdar, *et al.*, 1990) have been reported to have anti-inflammatory agents that might protect inflammatory lesions in brain and involved memory improvement activity.

Immunosuppressant drugs have produced a cognitive impairment is associated with degeneration of hippocampal neurons histopathologically. Since alteration of immune function affects learning and memory, it was hypothesized that immunostimulant drugs improves learning and memory. The constituents of Saraswatarishta such as *Withania somnifera, Tinospora cordifolia, Terminalia chebula, Piper longum, Pueraria tuberose* and *Asparagus racemosus* have been proved for cognitive enhancement. The probable mechanism of cognitive enhancement by Saraswatarishta could be by immunostimulation and increasing the synthesis of acetylcholine which is an important neurotransmitter in learning and memory process (Bisset and Nwai, 1983). This central action could be due to supplementation of choline by *Tinospora cordifolia* which is an important active constituent of Saraswatarishta (Toes and Mohammed, 1999).

Oxygen free-radicals are responsible for the development of Alzheimer's disease (Berr *et al.*, 2002). The antioxidant rich diets improved cerebellar physiology and motor learning in mice (Bickford *et al.*, 2000). *Bacopa monnieri, Withania somnifera, Terminalia belerica Terminalia chebula Tinospora corifolia, Foeniculum vulgare* (Choi and Hwang, 2004) *Acorus calamus* (Manikandan and Devi, 2005), *Zingiber officinale, Asparagus racemosus and Elettaria cardamomum* (Hinneburg *et al.*, 2006) are ingredients of Saraswatarishta, have been reported to possess antioxidant property, this resulting in reduced brain damage and improved neuronal function. Thus a combination of antioxidant, anti-inflammatory and immunostimulant action could all be leading to the net memory-enhancing effect of Saraswatarishta.

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