EXPERIMENTAL PROTOCOLS FOR MEDHYA ACTIVITY OF AYURVEDIC DRUGS

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**ABSTRACT**

In Ayurveda Medhya refers to one which enhances Medha i.e power of perception and retention of knowledge. It is a type of buddhi. Drugs which increase the Medha or memory power are known as Medhya dravyas. Memory can be co-related to Medha in Ayurveda. Medhya being a very vast concept in Ayurveda needs to be understood well. Memory can be defined as the impression of thoughts, experience, feelings, sensations, ideas and knowledge etc. To test this Medhya guna we take help of the experimental protocols. The Experimental Protocols are pre-defined written procedural method in the design and implementation of experiments. These can be carried out in two ways i.e in vitro and in vivo methods. To assess the Medhya activity usually in vivo method is being carried out, where in the animal models are being used to assess the Medhya activity. The various protocols are Morris Water Maze test, Elevated Plus maze test, Cook’s Pole climbing test, Gross Behaviour test etc. many such other tests needs to be utilised to assess the various activities in animal models. This article is concerned only with the Medhya activity of the Ayurvedic drugs, to assess the Medhya guna by using these tests. In this article an attempt has been made regarding the various experimental protocols which can be used to assess the Medhya activity.

**Keywords:** medhya, medha, experimental protocols, morris water maze test, elevated plus maze,

**INTRODUCTION**

Medhya refers to one which enhances Medha (the power of perception and retention of knowledge) i.e Memory power. It is correlated to Dhi, Dhriti and Smriti of a person. Medha is a type of buddhi, which either grasps or retains knowledge. Drugs which increase the Medha are known as Medhya dravyas \(^1\) according to Charaka Chikitsa sthana first chapter explains as; Medhyani chaitani rasayanani medhya visheshena cha shankhpushpi

The paste of Shankhpushpi(Convolvulus pluricaulis), juice of mandukaparni (Centella asiatica), powder of yashtimadhu (Glycyrrhiza glabra) mixed with milk, juice of guduchi (Tinospora cordifolia) along with its roots and flowers are described to have Medhya Rasayana activity. These increases longevity, cure diseases, promotes strength, agni, varna, swara to a person consuming this and is prescribed by many Ayurvedic practitioners. Memory can be defined as the registration, retention and recollection of experi-
ences, thoughts, feelings, sensations, ideas and knowledge. The hippocampus and cerebral cortex are key structures of memory formation. The Papez circuit is responsible for the memory formation. The pathway starts from; 1) Diffuse cortex to 2) Hippocampal cortex 3) Fornix 4) Mammillary bodies 5) Thalamus 6) Cingulate Bodies back to the diffuse cortex, this completes the circular circuit and provides continual reintegration of information. A wide variety of clinical syndromes like head trauma, convulsive disorders and neurodegenerative diseases manifest cognitive or memory dysfunctions. Cognitive and behavioural impairments are also shown by most common dementia in elderly. Although primary cause of these remains unclear, recent studies have suggested that free radicals produced during oxidative stress or inflammatory processes are pathologically important.

However, there has not been sufficient investigation to establish the biological effects of these drugs in improving cognitive deficits or memory disorders. Therefore it is necessary to evaluate the beneficial effects of these plants on learning and memory by means of proper animal learning model with various sample treatments. Currently the mainstays of pharmacological treatment for the cognitive deficits are donepezil, galantamine, rivastigmine and memantine, which are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use. Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders.

Protocol is a defined set of rules or guidelines and Experimental Protocols are predefined written method in the design and implementation of experiments. It is a plan or a procedure for carrying out a scientific experiment or a course of medical treatment. The FDA, Good Laboratory Practice Regulations Section 58.120 requires that each study has a written experimental protocol that clearly sets out objectives and methods for the test to be run. The institute for laboratory Animal research of the U.S National Academy of sciences has argued that animal research cannot be replaced by even sophisticated computer Models, which are unable to deal with the extremely complex interactions between molecules, cells, tissues, organs, organisms and the environment. Animals are used in research, when there is a need to find out what happens in the whole living body, which is far more complex than the sum of its parts. CPCSEA and IAEC permission is required to carry out any animal experiment. The necessities for the experimental studies are as follows;

a. For advanced scientific understanding; many basic cell processes are same in all animals and the bodies of animals are like humans in the way that they perform many vital function, to treat the diseases to understand how the body works.

b. As models to study disease; Humans and animals share hundreds of illness and consequently animals can act as models for the study of human illness, to study how disease affect the body.

c. To develop and test potential forms of treatment; animals are used to develop and test these potential therapies.

d. To protect the safety of humans; New medicines require testing because researchers must know both the beneficial and the harmful effects of a compound on a whole organism.

The various tests carried out system wise are as follows;
CVS - Anti arrhythmic activity, Calcium uptake binding, Anti-hypertensive activity
RS - Antitussive activity,
GIT - Emetic and Antiemetic activity, Anti-Obesity, Anti-Atherosclerotic
Urinary system - Diuretic and saluretic, Impaired Renal function, Uricosuric and Hypouricemic activity
In CNS - Sedatives, Anxiolytic, Antiepileptic, Anticonvulsant, Hypnotic, Psychotropic and Neurotropic activity, Neuroleptic, Antidepressant, Anti Parkinsonism, General Anesthesia, drug effect on learning and Memory, Immunomodulatory, Anti-Psychotic etc.
The tests can be carried out in two methods, viz in vitro method and in vivo method. In vitro methods for learning and memory are as follows;
1) In vitro inhibition of acetylcholinesterase activity in rat striatum.
2) In vitro inhibition of butyrylcholine esterase activity in human serum.
3) Ex vivo cholinesterase inhibition.
4) Molecular forms of acetylcholinesterase from rat frontal cortex and striatum.
5) Release of $^3$H ACh and other transmitters from rat brain slices.
6) Inhibition of respiratory burst in microglial cells/ macrophages.
7) Cultured neurons/astroglial cells.
8) Stimulation of phosphatidylinositol turnover in rat brain slices.
In vivo methods are as;
1) Inhibitory (passive) avoidance
2) Step down
3) Step through
4) Two compartment test
5) Uphill avoidance
6) Trail to criteria inhibitory avoidance
7) Scopolamine induced amnesia in mice
8) Active avoidance
9) Runway avoidance
10) Shuttle box avoidance (2 way shuttle box)
11) Spatial discrimination
12) Conditioned response
13) Olfactory learning
14) Visual discrimination
15) Morris water maze test
16) Elevated plus maze test
17) Cook’s pole climbing test
18) Gross behaviour.

Experimental protocols which are commonly followed to assess the Medhya activity in Ayurveda are.
1. Morris water Maze test
2. Elevated Plus maze test
3. Cook’s Pole climbing test
4. Gross behavior test

**MATERIALS AND METHODS:**
Schedule for experimentation;
Tests carried out for 24 days.
- First 1$^{st}$ - 6$^{th}$ day - training is given.
- Next (6 days) 7$^{th}$-12$^{th}$ days - Standard drug given to enhance the memory (Piracetum -150mg/kg). Piracetam has been claimed to improve ATP/ADP ratio in telencephalon, stimulate synaptic transmission and to have an antithrombotic effect. It is indicated in senile dementia and confusional states of old age, mental retardation and learning problems, cerebrovascular accidents etc.
- Next (6 days)13$^{th}$-18$^{th}$ day - Drug is induced for memory loss (Scopolamine -2 mg/kg). Scopolamine acts on CNS effect causing depression, amnesia, fatigue, drowsiness etc.
- Next (6 days) 19$^{th}$ - 24$^{th}$ day - Test drug is given.

The various tests are as follows;

Procedure; - Usually this protocol is carried out for all the tests initially in the beginning of an experiment. This is mainly
to see the adverse and toxic effect of the test drug in animals.
Test drug administered 1 hr before an experiment. Observations of behavioural changes are made every hour for 4 hrs (1, 2, 3 and 4hr). Rats are placed one by one in the center of the three concentric circles drawn on a rubber sheet diameter 7cm, 9cm and 13cm.
Application – Adverse and toxic effect of test drug.
The activities which would be recorded are;
a. ANS effects- Ptosis, Exophthalmos,  
b. CNS depression- Hypoactivity, Relaxation, Ataxia, Narcosis,  
c. CNS stimulation- Hyperactivity, Irritability, Tremors, Straub tail, Analgesia

2. Morris Water Maze test;
Developed and advanced by Richard Morris. From 1981- 1984, represents a more specific test of spatial learning based memory.
Apparatus:-
Maze (cylindrical black metallic pool of 170 cm diameter) filled with water, hidden Platform (10cm d) in maze, Skimmed milk to make the water opaque.

Procedure:- Pool is divided into 4 equal quadrants by two imaginary lines. An escape platform (10cm dm) is hidden 2cm below the surface of the water which is kept constant. During trial the rat is placed in one quadrant, all the four quadrants should be used for each session. 4 trials carried out for 6 days, each for 60 sec with a gap of 30 sec. After climbing the platform rat is allowed to wait there for 30 sec. Time taken to reach the platform is recorded as Escape latency.
Next day retention memory test is carried out. Platform is removed, rat is allowed to swim for 60 sec. Time spent in target quadrant, which contained the platform is recorded. Enhancement of cognition is reflected by a decrease in escape latency and increase in retention time.
Application - It is most widely used for learning and memory test rather than the anxiolytic activity.

3. Elevated Plus Maze Test;
Apparatus – Plus shaped apparatus with two covered and an open arm, each with an open roof, elevated 40-70 cm from the floor.

Procedure – Rat is placed on the plus maze in the open arm
Transfer latency (TL) is the time taken by the rat to move into any one of the covered arm. (As rats spends more time in closed arm normally). It is trained.
After causing amnesia, the rat spends time in open arm.

Test drug given, trial carried out, rat spends its time in covered arm. Time is noted during each trial. Retention of this learned task is examined 24 h after the trail.
Applications: Memory and learning, Anti-anxiety Model.

4. Cook’s Pole climbing test
Apparatus ; - An experimental chamber (25×25×25 cm) stainless steel floor grid, soundproof enclosure, Wooden pole 2.5cm diameter.

Procedure: - Rat is placed on the floor grid. Conditioned stimulus i.e buzzer signal given with a flash of light. Unconditioned stimulus i.e electric shock (0.1mA) delivered through floor for 45 sec. Avoidance response is climbing reaction time less than 10 sec only. Escape response is climbing after applying reaction time more than 10 sec. maximum each rat 5 trials given.

Recording transfer latency i.e ;-
Time at which the rat is placed on the floor grid, time at which rat would jump on to the pole, as a shock free zone, total time spent on pole, time taken to climb the pole are considered to determine learning and memory.
Application - Used to study cognitive function, mainly a response to stimuli during learning and retention.

RESEARCH UPDATES
1. Effect of *Convolvulus pluricaulis Choisy* on learning behavior and memory enhancement activity in rodents
Nootropic activity tested using Cook’s Pole climbing apparatus, passive avoidance paradigm and active avoidance tests used to test learning and memory. Two doses of ethanolic and ethyl acetate and aqueous fractions were given to separate groups of animals. Both doses showed improved learning and memory in rats. Doses significantly reversed the amnesia induced by scopolamine.

2. Effect of *Convolvulus pluricaulis Choisy* and *Asparagus racemosus Willd* on learning and memory in young and old mice a comparative evaluation
Both the drugs in a dose of 200mg/kg po showed significantly higher percent retentions than piracetam. But effect was more prominent with *Convolvulus pluricaulis Choisy* in old and young mice, here elevated plus maze test was used to assess the learning and memory in mice. Also the tissue prepa-
ration for microscopy of the brain was done to study the AChE activity.

3. Pharmaceutico analytical study of duration of sneha paka with special reference to Brahmi Ghrita and its effect on memory in albino rats

Brahmi Ghrita prepared in 3 different days and its analytical and experimental evaluation was done. 1 Group - one day paka, 2 Group – three day paka, 3 Group – five day paka. These samples were tested for organoleptic character, administered to albino rats and assessment of memory was done by Morris water maze test. Result and conclusion- For memory recall and short memory, enhancing effect = group 3 and 2 equally effective. Anti-Amnesic effect = All 3 groups were effective. In Scopolamine induced Amnesia = Group 2 statistically significant. Group 2 showed better results, in learning based memory.

4. Pharmaceutico Analytical study of Vacha Arka prepared by two different methods and evaluation of its Medhya effect

Control Group 1- normal diet and tap water 5ml/kg, Standard group 2- piracetam 150 mg/kg, Group 3 – Vacha arka A - (1:2) , Group 4 – Vacha arka B - (1:3). Tests performed- Gross behaviour, Mirror chamber, Morris water maze, behavioural test, anti-stress, anticonvulsant test etc were done. Group 3 better is in anti-anxiety, anti-convulsant. Group 4 showed better effect on learning and memory.

DISCUSSION:
• Hippocampus and cerebral cortex are key structures of memory formation

Hippocampus functional impairment results in the decline of learning. Memory establishment is equal to learning and vice versa.

• As the concept of Medha itself is vast in Ayurveda one needs to understand it first and then apply these tests and assess the Medhya activity

• To assess the Medhya, activity first the animal has to be trained followed by drug administration and the test is being carried out to check the memory and retention memory of the animal which is compared with the standard drug to see the effect of the Ayurvedic drug.

• The above mentioned tests used, especially in vivo method are very time consuming and are done under the guidance of an expert and can be used to carry out comparative studies.

• As Morris water maze test is easy to perform, simple and convenient usually it is being used in Ayurvedic research to assess the Medhya activity of a drug. As mentioned in the research updates, the complementary sciences use elevated plus maze, Cook’s pole climbing tests etc.

• Along with the in vitro studies like tissue preparation for microscopy of brain to study the acetylcholinesterase (AChE) activity, preparation of incubation media and histochemical detection of AChE etc. This gives the detailed description about pharmacodynamics of the drug on brain cells in relation with cognition.

• Usage of single method alone will not be enough to prove the Medhya activity. One has to carry out detailed research at the tissue level to prove the specific action of the drug, providing it a detailed scientific data.

• Advanced new techniques needs to be adopted in Ayurveda researches to have a strong scientific valid documentation and to stand apart to prove the pharmacodynamics and pharmacokinetics of a drug to the world, increasing the quality of Ayurvedic research making it more valid for further studies.
• Selection of a proper in vitro study with a in vivo protocol should be emphasized to know the drug action in detail, also sufficient investigation to establish the biological effects of such drugs in improving cognitive deficits or memory disorders will help serve the purpose.

CONCLUSION

Experimental protocols are the basis for pharmaceutical testing, used to plan an experiment and to standardize a laboratory method. All the tests explained are useful but Morris water maze test of learning and memory is most widely used in Ayurvedic research. Principle and working of all these tests are similar, simple, convenient and easy to understand; hence used to determine the Medhya effect in animals especially rats, whereas the in vivo studies are costly and they require sophisticated instruments. For both the studies a skilled person is required to perform and are time consuming. Experimental study serves as guideline for the clinical trials. Now the need is to utilize proper protocols in vitro method and correct selection of in vivo study to assess the Medhya activity of Ayurvedic drugs in research will help to understand the action of a drug in detail giving it a detailed scientific data. The exact mechanism of memory strengthening effect of the drug needs to be explored in the future studies.

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